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Award Number: DAMD17-96-1-6297

TITLE: Evaluation of the Health Risks of Embedded Depleted Uranium (DU) Shrapnel on Pregnancy and Offspring Development

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REPORT DATE: May 2001

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 2001	Final (23 Sep 96 - 30 Apr 01)

4. TITLE AND SUBTITLE

Evaluation of the Health Risks of Embedded Depleted Uranium (DU) Shrapnel on Pregnancy and Offspring Development

5. FUNDING NUMBERS

DAMD17-96-1-6297

6. AUTHOR(S)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

The results of studies conducted with female rats implanted with depleted uranium pellets showed that significant levels of uranium could be measured in the rat urine and tissues sampled upon necropsy. Despite the significant uranium levels, especially in the kidney, this methodology did not yield the renal toxicity that could have been expected from the scientific literature. Most likely this is due to the method of administration, a constant uranium supply from an implanted pellet in the rat leg.

Of specific interest to any female soldiers who may, in the future, find themselves in the position of some of our male Gulf War veterans, with DU shrapnel injuries, are the data reflecting the lack of a significant impact of the DU pellets on the maternal and litter parameters. Subsequent studies also showed no strong influence of the imbedded DU on fertility parameters, even when the pellets remain in the maternal rat for an extended period.

14. SUBJECT TERMS	15. NUMBER OF PAGES
depleted uranium; pregnancy	79
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

EVALUATION OF THE HEALTH RISKS OF EMBEDDED DEPLETED URANIUM (DU) SHRAPNEL ON PREGNANCY AND OFFSPRING DEVELOPMENT

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EVALUATION OF THE HEALTH RISKS OF EMBEDDED DEPLETED URANIUM (DU) SHRAPNEL ON PREGNANCY AND OFFSPRING DEVELOPMENT

Introduction

Background and Significance

Natural uranium (U) consists of three isotopes: ^{238}U (99.276%), ^{235}U (0.718%), and ^{234}U (0.0056%). During the uranium enrichment process two products are produced, "enriched uranium" and "depleted uranium" (DU), that contain different relative ratios of these three isotopes. Enriched uranium contains the higher amount of the fissionable isotope ^{235}U and is used for nuclear reactor fuel and nuclear weapons. DU has a lower ^{235}U content. The DU used by the United States in kinetic energy penetrators is alloyed with titanium (0.75% by weight) to increase its tensile strength and to retard oxidation[21]. Approximately 50% of current U.S. antitank weapons contain DU penetrators, and most of the Abrams main battle tanks are armored with DU. During Operation Desert Storm, at least 40 tons of DU munitions were fired by the U.S. Army and Air Force (Daxon, personal communication). Unfortunately, during this conflict, a number of U.S. military personnel were wounded by DU shrapnel[7, 8, 16]. Many of these fragments were not removed because the removal procedure would produce excessive tissue damage. Shrapnel fragments as large as 20 mm have been noted in patients. Ongoing uranium bioassays in these men indicate that uranium was present in the urine well in excess of natural background, up to 30 µg U/l of urine[20, 31].

The long-term health impact of leaving these radioactive and chemically toxic fragments in place is unknown. Further, military roles are changing significantly and the female soldier now plays a vital part in many combat scenarios. It is therefore important to include female soldiers among those that might be injured by DU shrapnel. Consideration also must be given to the potential harmful effects of *in utero* exposure to embedded DU shrapnel fragments on fetal and offspring development. This is important because animal research has shown that females are less sensitive to the effects of uranium than are males[44]. Thus, while the female may tolerate a greater dose of DU with no adverse effects, the dose may still lead to detrimental effects on the offspring.

Uranium toxicity

Although the toxicity of embedded DU is unknown, numerous studies have addressed the consequences of inhalation, ingestion and parenteral administration of other forms of uranium[9, 11, 18, 22, 26, 27,28, 30, 34, 36, 37, 38, 42, 43, 48]. After uranium is absorbed, it circulates in the blood as the uranyl ion forming uranium-carbonate and uranium-albumin complexes. As the uranium-carbonate complex passes through the kidney, it is filtered rapidly by the glomeruli where 60%-80% of absorbed uranium is excreted in the first 24 hours after acute exposure. The uranium that is not excreted is reabsorbed by the proximal tubules where it produces significant toxic effects. Uranium also enters the bone, where it competes with calcium to form complexes with phosphate ions, thus becoming part of the bone matrix[5, 14, 17, 35]. This bone matrix then serves as both a long- and short-term storage site from which uranium has been shown to be slowly released back into circulation[23, 47]. The liver,

muscle, and kidney are other major sites of uranium deposition, with a possible long-term storage mechanism in the kidney[23, 47].

Acute morphological and biochemical changes of the kidney result from uranium exposure[9, 25, 29, 35]. Changes in the glomerular epithelial architecture[24], and cellular necrosis in the proximal tubules near the corticomedullary junction of the kidney have been reported in experimental animals after acute uranium exposure[4, 18, 19]. In addition, polyuria, enzymuria, glucosuria, and increased excretion of amino acids have been reported[9, 10, 25, 49]. Acute renal failure can occur following exposure to high doses of uranium[35, 45]. Environmental stressors such as restricted diets or changes in housing conditions have been shown to significantly enhance uranium toxicity[1, 6].

Uranium-induced Fetal and Developmental Toxicity

In utero exposure to uranium has recently been shown to produce both fetal and developmental toxicity. For example, administration (s.c.) of uranium in the form of uranyl acetate dihydrate (0.5-2.0 mg/kg/d) to gravid (pregnant) mice from gestational days (GD) 6-15 leads to significant decreases in both maternal weight gain and fetal body weights at GD 18[3]. Soft tissue and skeletal examination of the fetuses also revealed a significant increase in the occurrence of renal hypoplasia in all uranium-treated groups. Skeletal anomalies in these mice included bipartite sternebrae, dorsal hyperkiphosis, and incomplete ossification of several bones. Similar skeletal malformations were also seen following daily oral administration of uranyl acetate dihydrate (5-50 mg/kg/d) in gravid mice during the same period of gestation[13].

While the above results examined the effects of uranium on prenatal development, several studies have been conducted to evaluate the effects of uranium on postnatal development (from birth to age 21 days)[12, 39]. Significant decreases in body weight and body length in the offspring of mice treated with 25 mg/kg/d for 14 days prior to mating have been reported[39]. There were also significantly more dead young per litter at this uranium dose at both birth and day 4. Uranyl acetate given orally to gravid mice from GD 13 to 21 days following parturition led to a significant increase in offspring liver weights in all the uranium treated groups (5.0-50.0 mg/kg/d), and decreased mean litter size on day 21 in the highest dose group (50 mg/kg/d). However, developmental parameters such as pinna detachment, incisor eruption and eye opening were unaffected[12].

Unfortunately, uranium levels in the dam, fetus, or placentae were not measured in any of these fetal and developmental toxicity studies. In order to determine the effects of embedded DU on a developing fetus, it is important to know the *in utero* uranium exposure level, though little work has been done to examine the cross-placental transfer of uranium[2, 15]. While there are distinct anatomical differences between the rodent placenta and the human placenta, little correlation has been shown between the anatomic classification of the placenta and the transfer of xenobiotics between mother and fetus[46]. In rodents and primates, the placenta may act as a barrier, limiting or preventing many toxicological insults to the fetus. This does not appear to be the case with uranium. When ^{233}U was administered intravenously to pregnant rats, almost identical levels of uranium were found in the placenta and fetus[41], indicating little discrimination for uranium by the placenta. The soft tissue levels of uranium in 19- to 20-day-old fetuses were equal to or greater than the maternal

liver concentrations. Immature bone also exhibited a greater deposition of uranium than did the adult bone[15].

The effects of depleted uranium in the male rat have been studied within the institute. This project aimed to determine the toxicity of depleted uranium in the female rat and its effects on the female rat's ability to become pregnant and deliver healthy litters.

Kidney Toxicity and Uranium Distribution

Materials and Methods

Subjects

Sprague-Dawley rats(Charles Rivers) weighing 250-300 g were used. Rats were maintained in an AAALAC-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23). Upon arrival, rats were quarantined and screened for diseases. Except during urine collection, all animals were housed in plastic microisolator rat cages with hardwood chips as bedding. Commercial rodent chow and acidified water (pH 2.5, using concentrated HCl) were provided *ad libitum*. Rats were on a 12-hour light/dark cycle.

Depleted Uranium and Tantalum Pellets and Surgical Procedures for Pellet Implantation

Depleted uranium pellets (1 mm diameter x 2 mm long) were obtained from the Oak Ridge National Laboratories, Oak Ridge, TN. Tantalum pellets (1 mm diameter x 2 mm long) were obtained from Alfa Products, Ward Hill, MA and were the heavy metal control. Before the implantation surgery, the depleted uranium and tantalum pellets were cleaned and sterilized. Anesthesia was induced with ketamine hydrochloride (80 mg/kg) in combination with xylazine hydrochloride (4 mg/kg) and given i.p. in a 0.5-ml bolus, using a 25-gauge needle. The surgical sites were then shaved and cleansed with betadine. Pellets were implanted in each biceps femoris muscle spaced approximately 15 mm apart on the lateral side of each thigh. Implantation was accomplished by placing the pellet in a 16 gauge needle, putting a specially designed plunger inside that needle, pushing the needle into the rat muscle, then depressing the plunger. This forced the pellet out of the needle and into the rat muscle.

Dose

Six doses, with 8 animals per dose, were used in this study: 32 tantalum pellets, 16 depleted uranium pellets and 16 tantalum, 20 depleted uranium and 12 tantalum, 24 depleted uranium and 8 tantalum, 28 depleted uranium and 4 tantalum and 32 depleted uranium pellets (10). At all times, each rat had a total of 32 pellets implanted in order to keep the size of the implantations approximately equal in all surgery rats.

Urine and Blood Collection

Blood and urine samples were collected and analyzed for uranium levels and biological markers of kidney function. To assess whether embedded depleted uranium pellets resulted in acute

kidney toxicity, blood and urine samples were taken on 14, 28, 42, 56, 70, and 84 days after implantation surgery and assayed for indices of nephrotoxicity.

To safely collect blood samples, rats were immobilized by placing them in a Plexiglas restrainer. During each collection, approximately 0.3 ml of blood was obtained from the tail vein using a 22-gauge needle. Plasma and the red blood cells was separated by centrifuging for 5 min at 3,000 X g. The plasma was analyzed for uranium levels and biochemical indices of kidney toxicity.

Urine samples were collected by housing the rats in individual metabolism cages (23.5 cm diameter x 12 cm high) where they had continuous access to food and water. The rats were acclimated to the metabolic cages before the study began because naive exposure to these housing procedures has been shown to induce stress in the animals and to increase the toxicity of uranium.

A 24-hour urine collection sample was obtained from each rat and the volume recorded. Rats in the preliminary study produced 10-20 ml urine in a 24 hour sampling period. Care was taken to prevent contamination of the urine with food or feces. After collection, urine was filtered to remove any debris and stored in plastic containers at 4 C until analyzed. The metabolic cages were disinfected and decontaminated between each animal use. During the animal-handling periods, overt signs of behavioral toxicity and the overall appearance of the rats was noted.

Assessment of Uranium on Kidney Function

Measurement of urine volume and osmolarity, urine levels of NAG, LDH, glucose, total protein, creatinine, and blood levels of glucose, urea, and creatinine were used as indicators of kidney function. Osmolarity of the urine was measured with a vapor pressure osmometer (model 5100-B, Wescor Inc., Logan, UT). A Kodak Ektachem 700 Analyzer was used to determine serum and urine levels of creatinine, glucose, and urea. Total urine protein was measured with a dye-binding assay (Coomassie Blue, BioRad) sensitive down to 1 g. The activity of NAG was measured by the methods of Tucker et al. using 4-methylumbelliferyl-N-acetyl- -D-glucosaminide as the fluorescent substrate (excitation wavelength = 356 nm; emission wavelength = 446 nm). The dilution of the urine for this assay eliminates the effects of any inhibitors present. For LDH measurements, 1 ml of urine was dialyzed for 4 hours at 4 C with 1 liter of deionized water. LDH was quantitated with a colorimetric assay that measures a reaction product proportionate to LDH activity (Oxford Biomedical Research Inc.).

Determination of Uranium Distribution

Upon completion of the study the rats were euthanized and the tissues removed for analysis of uranium content. This was done using kinetic phosphorescence analysis (KPA). One kidney was also analyzed for any histological changes.

Results

Nephrotoxicity

Figure 1 shows the effect of depleted uranium pellets on the animal body weights. No significant effect of pellet number is seen on body weight. Bodyweight is a gross measurement of toxicity and from this data it indicates that even at a level of 32 pellets, the depleted uranium was not toxic to the female rat. Figure 2 indicates that the depleted uranium pellets had no effect on the urine output in all animals. This is also a good gross measurement of kidney function.

Figures 3-6 present the data obtained from analysis of serum for indicators of kidney toxicity. As the data indicates, no significant difference was seen in the potassium, urea nitrogen, glucose or creatinine levels in the blood. Figures 7-9 show that these same parameters when measured in the animal's urine again show no significant difference among the various depleted uranium doses. Figure 10 shows that creatinine clearance was not affected by the DU pellets. This number is calculated from the equation: $C_c = U_c * V / P_c$ where C_c is creatinine clearance, U_c is urinary creatinine, P_c is plasma creatinine, and V is the urine volume.

Figures 11 and 12 represent the results of urine analysis for the enzymes LDH and NAG. Both of these enzymes show no significant differences among the depleted uranium groups.

Figures 13-15 show that osmolarity, pH and protein levels of the urine are all unaffected by the depleted uranium pellets.

Histopathology, conducted by the pathology department of AFRRI, indicated no treatment-related histological changes in the kidney following 84 days of the implanted DU pellets.

Uranium Distribution

Uranium levels in the tissues and blood removed at the completion of the 84 days of pellet implantation are presented in Figures 16-29. These same data are also depicted in Figure 30, with all the tissues combined. Measurable levels of uranium were detected in all of the tissues analyzed. Uranium levels in the urine collected on days 14, 28, 42, 56, 70 and 84 post-implantation are presented in Figure 31. Although a dose-response relationship appears to be evident in several tissues/fluids sampled, the great variability that is usually seen with these samples do not allow for a significant dose-response effect.

Conclusions

The assays conducted provide a broad spectrum of measures of kidney toxicity. Many of these substances have been shown to be very sensitive biomarkers of acute uranium toxicity.[9,29] Urinary enzymes are sensitive noninvasive markers of toxicity primarily in the kidney tubules.[40] NAG is a lysosomal enzyme found in proximal renal tubule cells. LDH is a cytosolic enzyme of the tubular epithelium. Previous research has shown LDH, and to a lesser extent NAG, increased following uranium exposure.[9,29] In our study, neither of these enzymes was significantly altered by the depleted uranium, indicating no changes in the tubules of the animals' kidneys.

Although urine volume and osmolarity can vary greatly with fluid intake, these measures provide physical indicators of renal function. For example, kidney failure drastically decreases urine volume, while moderate renal insufficiency can increase urine output. Osmolarity can reflect the ability of the kidney to concentrate (or dilute) the urine. A transient increase in urine volume has been shown to occur with acute uranium toxicity.[29] Lack of changes in these measurements in our study indicates that renal function has not been significantly altered due to the uranium treatment.

A small concentration of protein is normally present in the urine. Increases in total urine protein could result either from glomerular leakage or failure of tubule reabsorption. The appearance of protein in the urine has been reported with acute uranium toxicity.[29] Our experiments found no alterations in urinary protein levels, indicating that the glomerular filtration and tubular reabsorption mechanisms are not altered by the depleted uranium implantations.

Appearance of glucose in the urine occurs when the tubule reabsorption maximum from the filtrate is exceeded. This can occur with hyperglycemia or with a decrease in tubular reabsorption capacity. Measurement of both urine and plasma glucose helps to distinguish between these two possibilities. Glucose is one of the most sensitive indicators of uranium-induced nephrotoxicity[9,10] Nephrotoxicity is indicated where there is increased glucose detected in the urine but no concurrent increase found in the plasma.

All these measures were used together as indicators of kidney toxicity. Interpretation of these data, along with the lack of any noted histopathological changes, lead to the conclusion that while previous experiments have shown that uranium exposure can alter the kidney structure and functioning, uranium exposure to female rats from implanted depleted uranium does not adversely affect the kidney. This is despite the fact that uranium levels in the kidney equal or exceed the level set by the Nuclear Regulatory Commission for kidney damage. The lack of kidney toxicity seen due to uranium certainly differs from what is reported in the literature. This could be due to the uniqueness of several aspects of our experimental design. The chemical form of uranium is different in these studies than the uranyl nitrate or acetate used in many of the studies. Our route and time course of administration, chronic levels due to metal implantation, differ from the acute exposures via injection or drinking water. Chronic inhalation of uranium dioxide in rats has also produced no signs of renal toxicity. It is possible that the chronic route of exposure allows for a mechanism of tolerance to develop that prevents the renal toxicity often seen with acute exposures.

The uranium distribution data held few surprises given the data that was obtained within the institute by Dr. Terry Pellmar. Those results were obtained in the male rats while these current data are from female rats. The female rats, as with the male rats, show very high levels of uranium in the kidney, marrow bone, teeth and muscle proximal to the DU pellets location.

Litter Effects and Fetal Uranium Distribution

Materials and Methods

Subjects

Sprague-Dawley rats were used under the same conditions as previously described. Pellet implantation procedures were also the same as in the first study. Ten female rats were assigned to each dosing group and were bred to untreated male Sprague-Dawley rats.

Dose

Five doses were used in this study: Non-surgery control (N=10), 12 tantalum pellets (12), 4 DU pellets and 8 tantalum (11), 8 DU and 4 tantalum (11) and 12 DU pellets (10). At all times, any rat receiving pellets always had a total of 12 pellets implanted in order to keep the size of the implantations approximately equal in all surgery rats.

Prenatal Tissue Collection

Experimental females were housed with non-treated male rats with two females in each male's cage. Gestational Day (GD) 0 was determined by the presence of sperm in the vaginal washing. At this time the females were removed from the males' cages and housed individually. From GD 0 until GD 20, pregnant rats were monitored daily for weight gain, food intake and water intake. The parameters were used as measures of maternal toxicity of the DU pellets. On GD 20, the dams were euthanized. Dams were immediately cesarean sectioned, and the uterine horns removed. Fetuses were dissected out, and all the placentae for that litter collected. The uterine horns were examined for any resorption sites. Litters were examined, and a record made of (1) total number of fetuses, (2) number of viable fetuses, (3) sex ratio, and (4) any overt signs of teratological effects. All offspring of the litter were analyzed for uranium levels. The placentae from all pups were collected and pooled for uranium analysis for each litter. One male and one female pup separated out and used for analysis of whole fetus. The rest of the litter was used for determining uranium tissue levels. Quickly the liver and kidneys were dissected out of these pups. These tissues were pooled for the entire litter, homogenized, and analyzed for uranium content.

Results

Maternal and Litter Effects

Tables 1 and 2 present the data on the effects of the DU levels on maternal and litter parameters. From these data, there appears to be no effect of the DU on maternal parameters such as: maternal food and water intake, weight gain during pregnancy, and time-to-pregnancy. Furthermore, the litter parameters such as: number of pups, number of males vs females, and fetal weight were also not affected by the various levels of DU. The DU pellets did not adversely affect the ability of these rats to breed, or for them to maintain the pregnancy until the day of euthanasia. All litters were examined for any overt signs of teratology, and none were noted.

TABLE 1

Effects of Depleted Uranium on Maternal Parameters					
Variable	No Surgery	0 DU	4 DU	8 DU	12 DU
# Dams Bred	16	16	13	17	14
Days to Pregnancy (± SEM)	3.9 (±1.76)	2.08 (±0.23)	3.36 (±1.11)	4.36 (±1.42)	4.9 (±1.97)
Mean Weight Gain (g)	133.79 (±8.13)	138.33 (±6.49)	143.26 (±4.69)	138.75 (±4.38)	145.22 (±6.89)
Mean Food Intake (g)	23.44 (±0.67)	24.51 (±0.68)	24.27 (±0.52)	23.67 (±0.79)	24.71 (±0.71)
Mean Water Intake (ml)	43.85 (±2.59)	44.45 (±2.60)	46.33 (±2.10)	48.10 (±1.91)	44.84 (±1.50)

TABLE 2

Effects of Depleted Uranium on Litter Parameters					
Variable	No Surgery	0 DU	4 DU	8 DU	12 DU
Total # Fetuses	13.8 (±.79)	13.5 (±.78)	14.8 (±.54)	15.5 (±.53)	15.0 (±1.09)
# Males	6.6	6.3	8.5	8.7	7.5
# Females	7.2	7.2	6.3	6.8	6.5
# Non-Viable	0	1	1	2	0
Average Pup Weight	3.60 (±.20)	3.16 (±.09)	3.66 (±.27)	3.39 (±.09)	3.38 (±.08)

Uranium Distribution

Figures 32 and 33 show the placental and whole fetus uranium levels. Comparison of these results by a correlation trend test indicates that uranium accumulates in these tissues in an increasing fashion as the maternal DU dose increases. There appears to be a ten-fold decrease in the levels within the whole fetus when compared to the placental tissue. The levels in the fetus do exhibit a dose-response relationship as determined by a correlation trend test.

Conclusions

These results show a dose response effect on uranium levels in the whole fetus. These data indicate no effect of the DU pellets on any of the maternal or litter parameters measured. Uranium implantation did not impact the ability of the dams to become pregnant or carry the litter to term, the number of viable and non-viable pups or the average weight of the pups.

Effect of Time Following Implantation on Litter and Reproductive Effects and Uranium Distribution in Pregnant Rats

The results of Dr. Pellmar's study led us to be concerned with the length of time the pellets should remain in the female rat prior to breeding. Initial experiments operated under the assumption that once the incision was healed the female rats should be bred immediately with the non-implanted male rats. Dr. Pellmar's data indicate that at time points of 6, 12 and 18 months post-implantation, the uranium levels accumulating in the male rat are still increasing. Given this, it was determined that a study should be conducted to examine the effects of an increased time between pellet implantation and breeding would have on the reproductive effects as well as the uranium distribution.

A group of rats were implanted with the highest dose of DU used to date, 32 pellets. For each DU rat there was a non-surgery control rat. These rats were further divided into one of 4 groups depending on the length of time the pellets would remain in the female prior to breeding: 2, 4, 6 or 9 months. Urine was collected from these rats on gestational days (GD) 6, 12 and 18. Analysis of this urine indicated, as seen in previous studies, no nephrotoxicity. The dams were euthanized on GD 20, and the pups removed, sexed, weighed, and observed for abnormalities. Maternal tissues were removed along with the placenta, whole fetus, fetal kidney, fetal liver and fetal brains. Procedures in this study did not differ from that previously described. The uranium distribution data, to the fetus and within fetal tissues, will provide information to us regarding the effects of the delay in breeding the female rats.

Results

Table 3 depicts the percentage of rats that were actually pregnant when euthanized on what was determined to be GD 20 from the positive sperm results during breeding. It is evident that over time, more animals that are sperm-positive on GD 0 are not pregnant at GD 20. This does not seem to be a treatment-related effect, as it is seen in the control group as well. One factor that is likely contributing to this effect is the age of the female rats. The stress of collecting urine from the pregnant dams may also have contributed to the early loss of pregnancies.

Table 3

Percentage Of Bred Animals Determined To Be Pregnant That Were Not Pregnant By Gestational Day 20				
Du Dose	Months Elapsed From Time of Pellet Implantation Until Breeding			
	2 Months	4 Months	6 Months	9 Months
No Pellets	100	100	75	0
32 Pellets	100	83	67	22

Figure 34 shows the litter sizes seen in the dams that were pregnant. With increasing time, it appears that the DU treatment yields litters of smaller size than is seen in the control group. Figures 35-39 show the uranium levels in the whole fetus, placenta, fetal kidney, liver and brain. Figure 40 presents these same data on one graph. It is evident that the highest uranium levels are detected in the placental tissue. Detectable levels are still evident in the whole fetus as well as the three fetal tissues that were analyzed, though with a few instances of very high variability within a group.

Tissue samples from all the bred females were also analyzed for uranium levels. Figures 41-52 show the uranium levels in tissues 2, 4, 6 and 9 months after implantation of the DU pellets. Over time, higher levels of uranium are usually detectable, especially in tissues such as teeth and bone, were the uranium is likely to be depositing, and the kidney, the route of elimination for the uranium.

Figure 53 shows the number of viable pups per litter in the control and high dose DU groups over the 4 time points. These initial data were disconcerting in that it appeared that as the DU pellets remained in the dam longer, she had smaller litters. This could be important information regarding the long-term effects of DU on fertility. The small number of pregnant animals, however, made these results suspicious.

This led to another study in which the dams were again implanted and then bred over several time-points. No urine was collected, so the confound of the stress from the metabolic cages was eliminated. The final time-point was changed from 9 months to 8 months and a tantalum control group was added, in addition to the non-surgical control. Figure 54 depicts the mean litter size from this study. Unlike Figure 53, this graph shows that only when the rats are bred 6 months after implantation is there a trend towards a smaller litter. As in the first study, the loss of pregnancies over time does not help strengthen the data, as the N becomes smaller with increasing time-points. Table 4 shows the percent of rats that although sperm-positive on GD 0, have no litters upon euthanasia at GD 20.

Table 4

Percentage Of Bred Animals Determined To Be Pregnant That Were Not Pregnant By Gestational Day 20				
Du Dose	Months Elapsed From Time of Pellet Implantation Until Breeding			
	2 Months	4 Months	6 Months	8 Months
No Pellets	100	100	67	33
32 Tantalum Pellets	92	92	71	33
32 DU Pellets	100	67	50	25

Figure 55 shows the number of resorptions that were recorded at the varying time-points. While the data appear to show an increase in resorptions with the DU group, the high variability makes this finding insignificant.

Figures 56 and 57 show the mean weights of the viable male and female pups obtained in this study. No clear cut effect of DU can be seen. This is an important finding because when a drug treatment does not lead to a significant decrease in birth weight in the pups, developmental delays are seldom seen in those offspring.

Figures 58-61 show the body weight gains post implantation of the pellets. The plot for the animals bred at 8 months, minor maternal toxicity can be seen as evidenced by the significantly lower weight gains in the rats implanted with DU pellets.

Overall Conclusions

The results of the studies conducted with female rats implanted with depleted uranium pellets showed that significant levels of uranium could be measured in the rat urine and tissues sampled upon necropsy. Despite the significant uranium levels, especially in the kidney, this methodology did not yield the renal toxicity that could have been expected from the scientific literature. Most likely this is due to the method of administration, a constant uranium supply from an implanted pellet in the rat leg.

Of specific interest to any female soldiers who may, in the future, find themselves in the position of some of our male Gulf War veterans, with DU shrapnel injuries, are the data reflecting the lack of a significant impact of the DU pellets on the maternal and litter parameters. Subsequent studies also showed no strong influence of the imbedded DU on fertility parameters, even when the pellets remain in the maternal rat for an extended period. While these data are not strong, the problem appears to be more related to the small N, which could be impacted by the age of the rats, than treatment-related. It should be noted, however, that measurable uranium was present in the placenta. And though at levels ten-fold lower than in the placenta, uranium was also detectable in the whole fetus tissue. It would seem that the full relevance of this exposure is still not elucidated. While no overt physical anomalies were noted in any of the litters, and there were not significant decreases in the treated pups body weights, there still remain some unanswered questions about the full impact of prenatal exposure, throughout gestation, to uranium from maternally implanted DU pellets.

Future Directions

As the investigator left the Armed Forces Radiobiology Research Institute in 1999 and is no longer in the laboratory research field, no further investigations appear to be planned involving maternal DU and *in utero* exposure problems. Were AFRRI interested in pursuing this line of research, a study into possible effects on mutagenicity or oncogene expression in the rats exposed *in utero* to DU, as has been seen in tissues from animals with direct exposure to DU[32,33]. The rat offspring are exposed to some amount of uranium, as evidenced by the graphs showing fetal levels of uranium, throughout development. It is quite possible that similar results could be seen with rats exposed in utero as has been seen with the DU implanted rats. From a neurobehavioral development standpoint, the lack of significant effects of the DU pellets on the birth weights of the pups is supportive of this form of DU exposure not affecting the offspring. If further studies were to be done, a more sensitive test such as the radial arm maze with and without pharmacological challenge may elucidate any changes in learning development following *in utero* exposure to implanted DU pellets.

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Figure 1

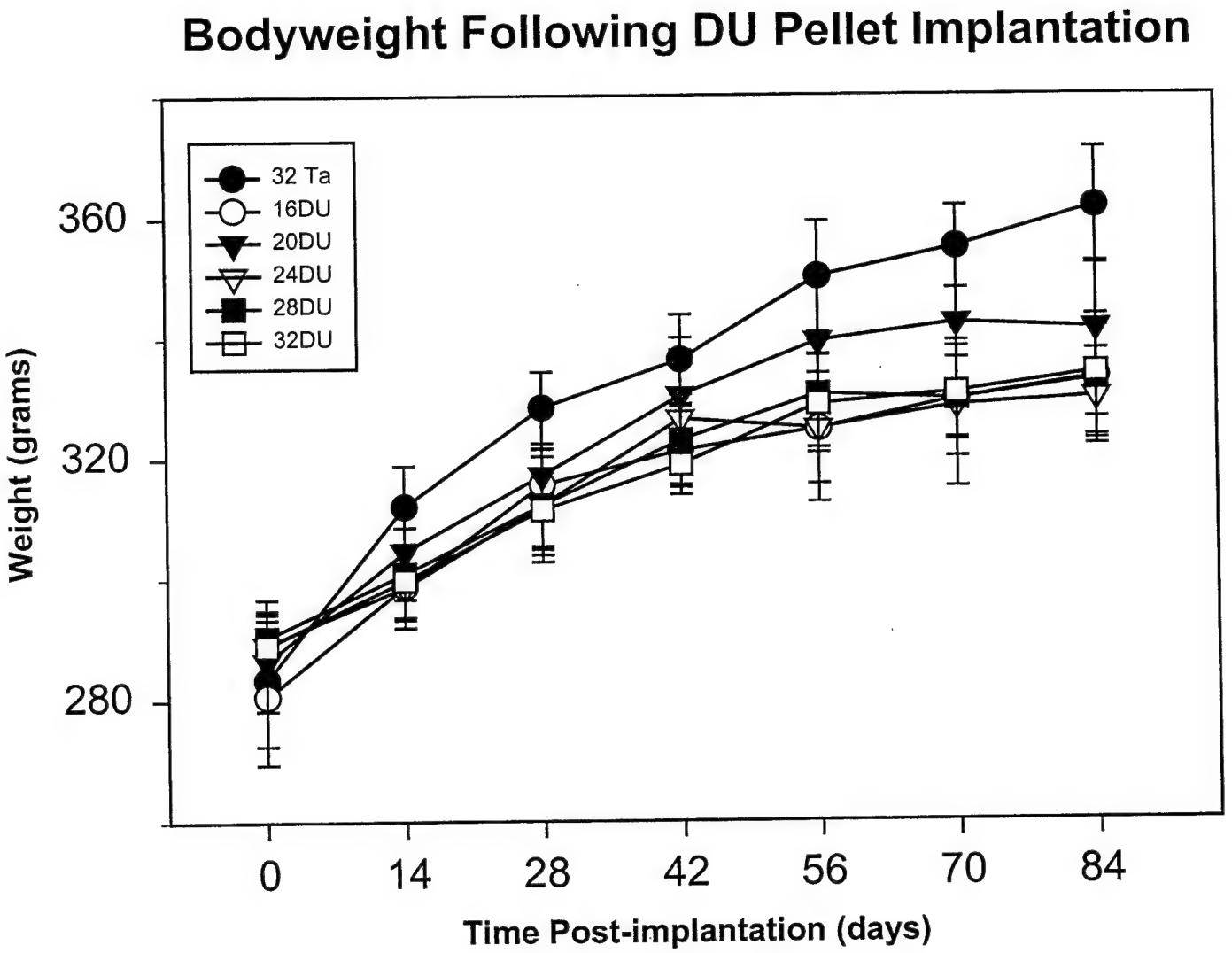

Bodyweight Following DU Pellet Implantation
32 Ta
16DU
20DU
24DU
28DU
32DU
360
320
280
Weight (grams)
0
14
28
42
56
70
84
Time Post-implantation (days)

Figure 2

Urine Output Following DU Pellet Implantation

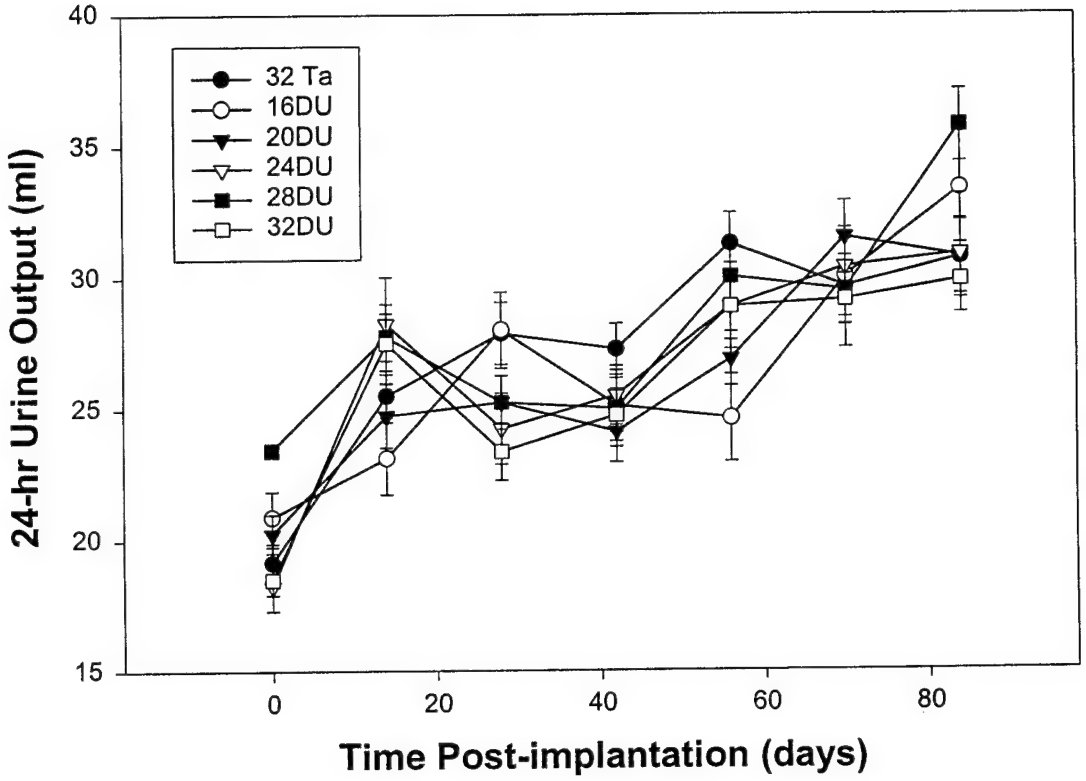


Figure 3

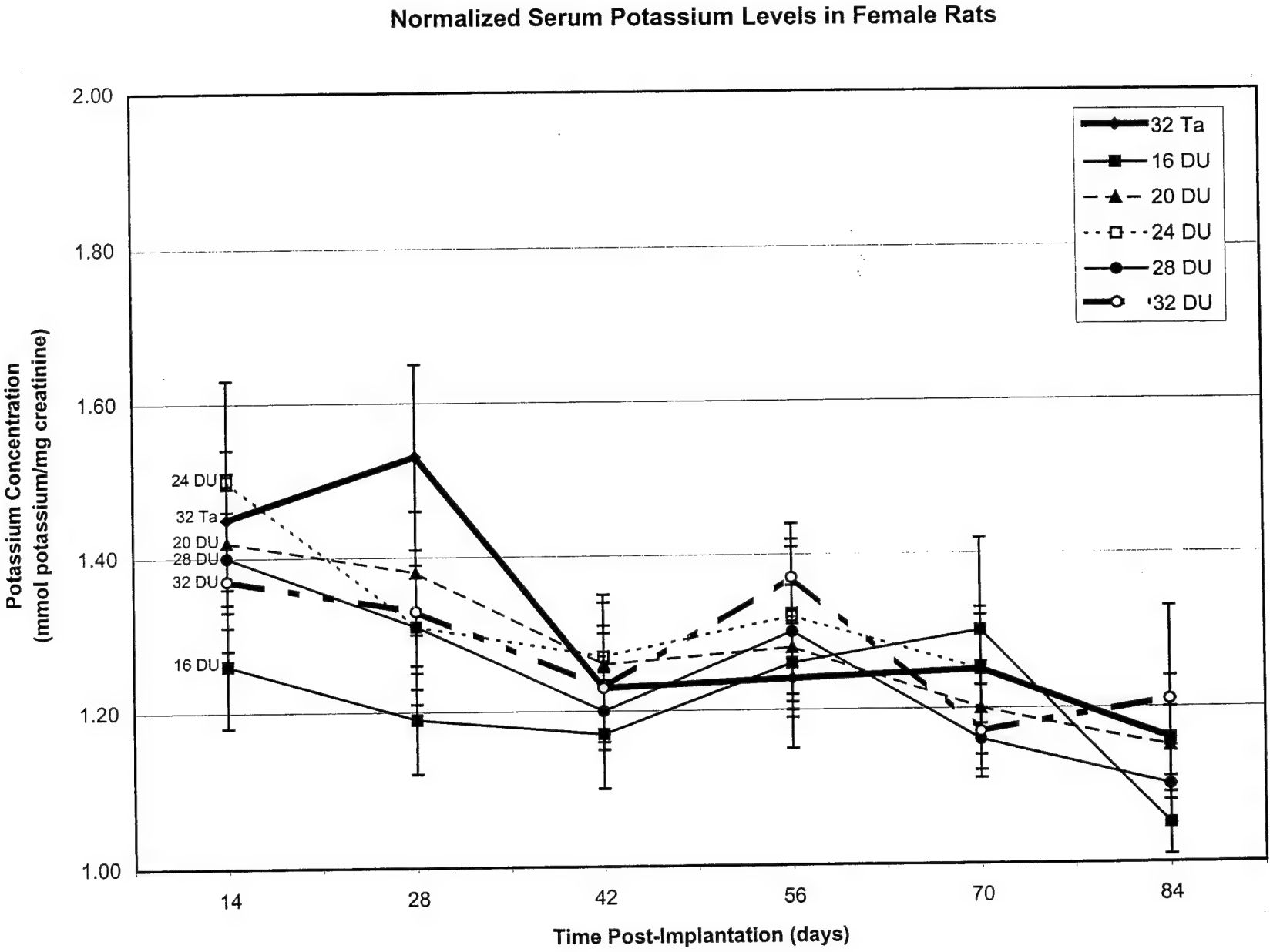

Normalized Serum Potassium Levels in Female Rats
Potassium Concentration (mmol potassium/mg creatinine)
2.00
1.80
1.60
1.40
1.20
1.00
14
28
42
56
70
84
Time Post-Implantation (days)
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
24 DU
32 Ta
20 DU
28 DU
32 DU
16 DU

Figure 4

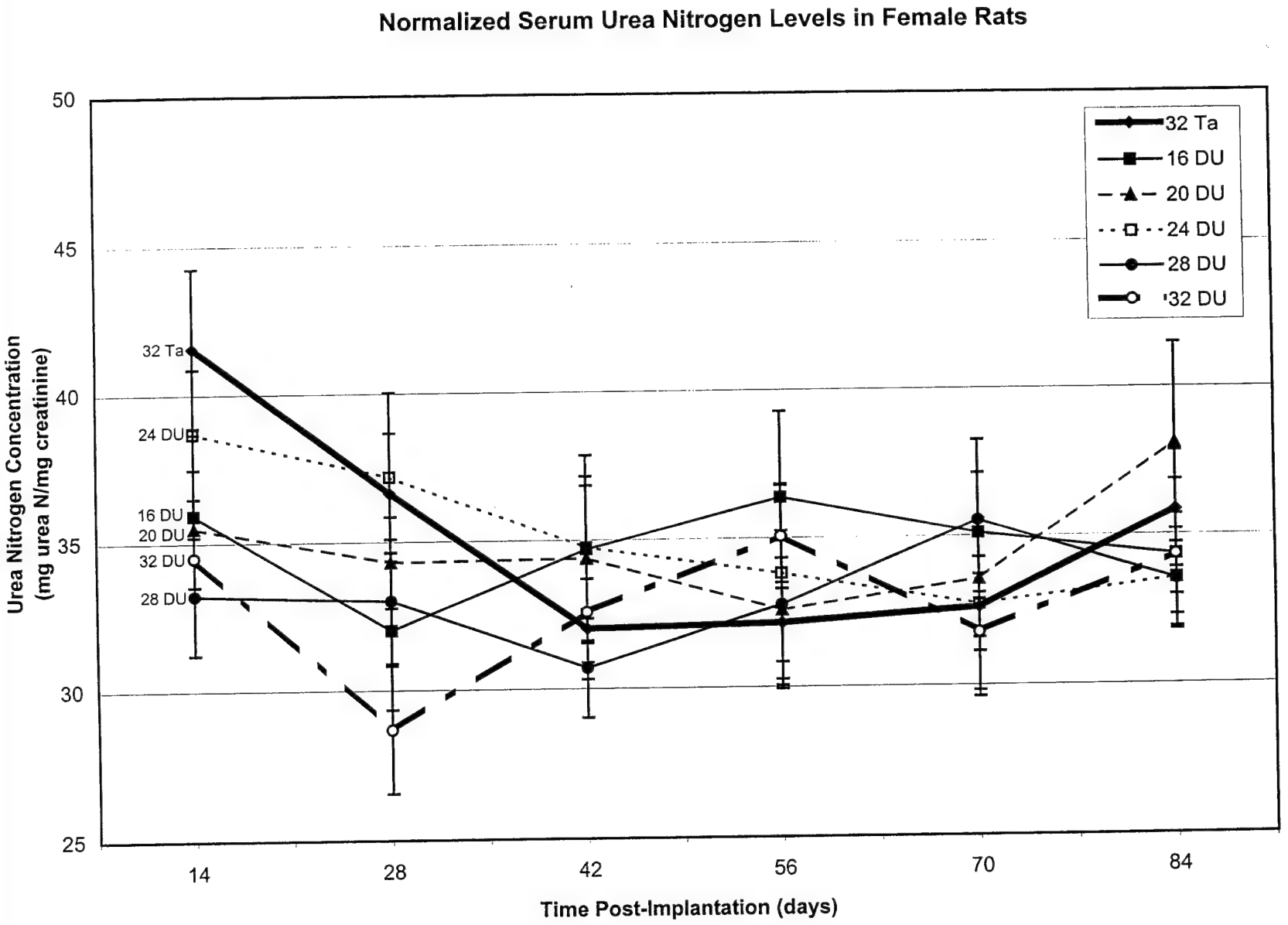

Normalized Serum Urea Nitrogen Levels in Female Rats
Urea Nitrogen Concentration (mg urea N/mg creatinine)
50
45
40
35
30
25
14
28
42
56
70
84
Time Post-Implantation (days)
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU

Figure 5

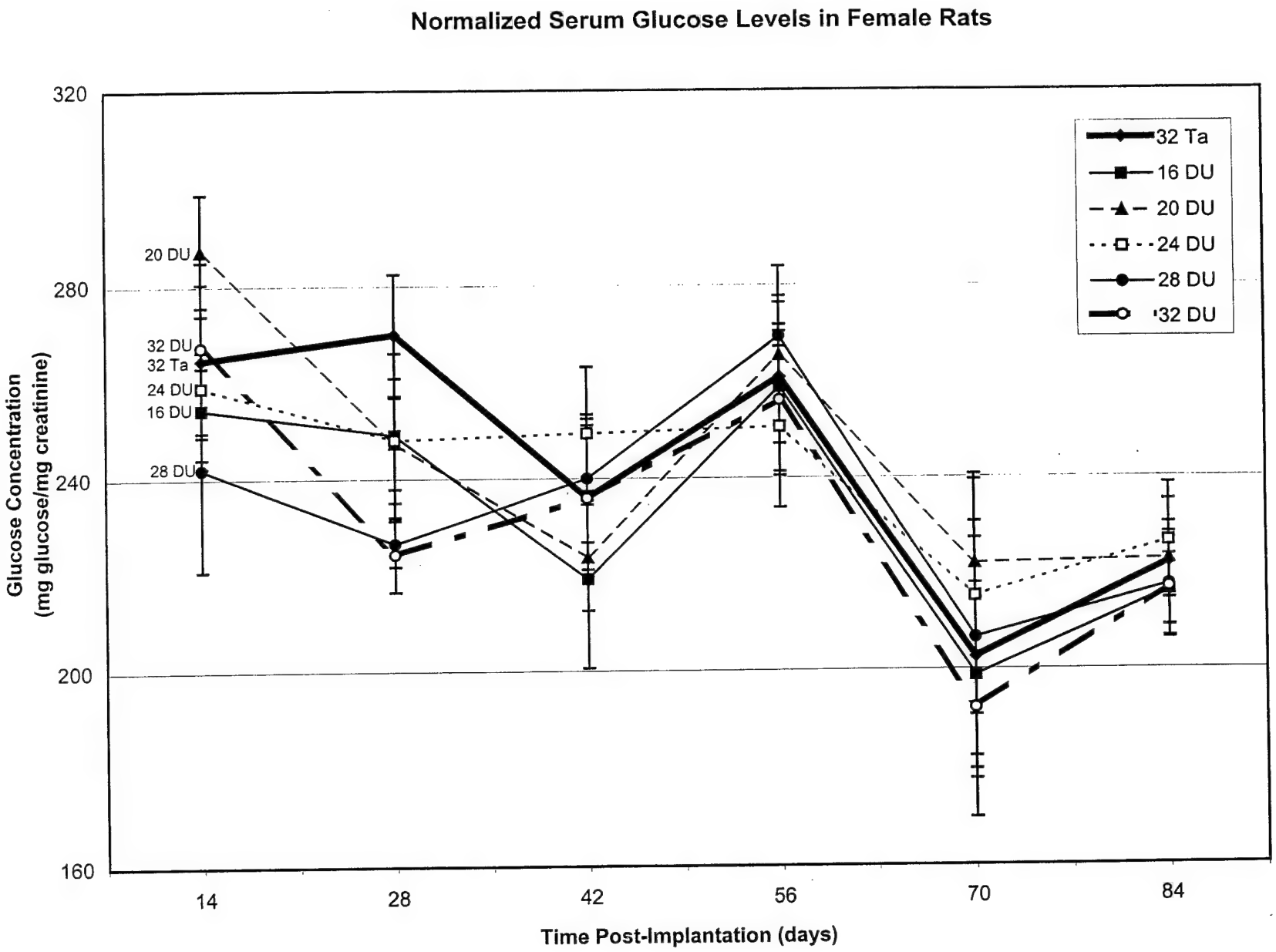

Normalized Serum Glucose Levels in Female Rats
320
280
240
200
160
Glucose Concentration
(mg glucose/mg creatinine)
20 DU
32 DU
32 Ta
24 DU
16 DU
28 DU
32 Ta
16 DU
20 DU
24 DU
28 DU
'32 DU
14
28
42
56
70
84
Time Post-Implantation (days)

Figure 6

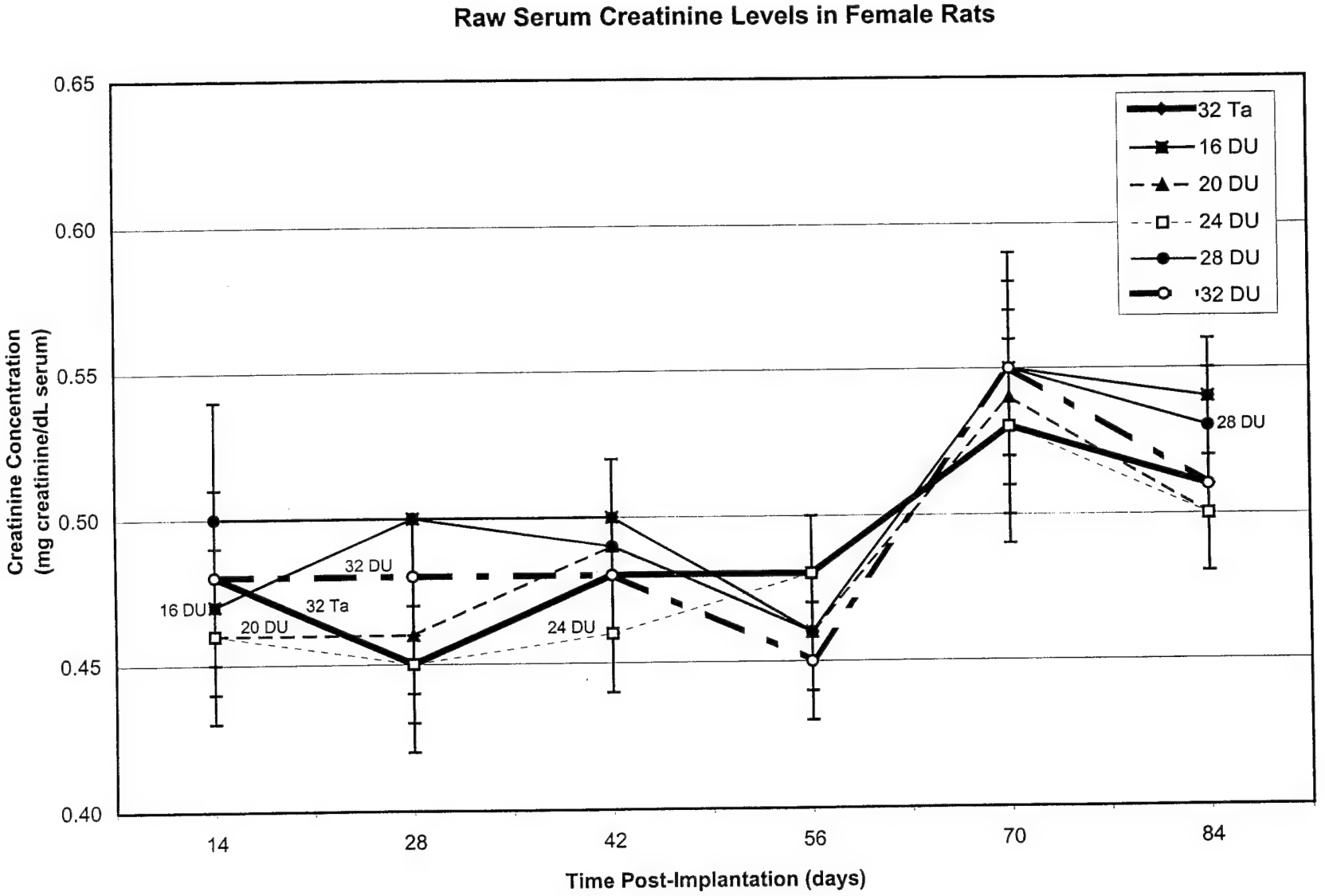

Raw Serum Creatinine Levels in Female Rats
Creatinine Concentration (mg creatinine/dL serum)
0.65
0.60
0.55
0.50
0.45
0.40
14
28
42
56
70
84
Time Post-Implantation (days)
32 Ta
16 DU
20 DU
24 DU
28 DU
'32 DU
32 DU
16 DU
32 Ta
20 DU
24 DU
28 DU

Figure 7

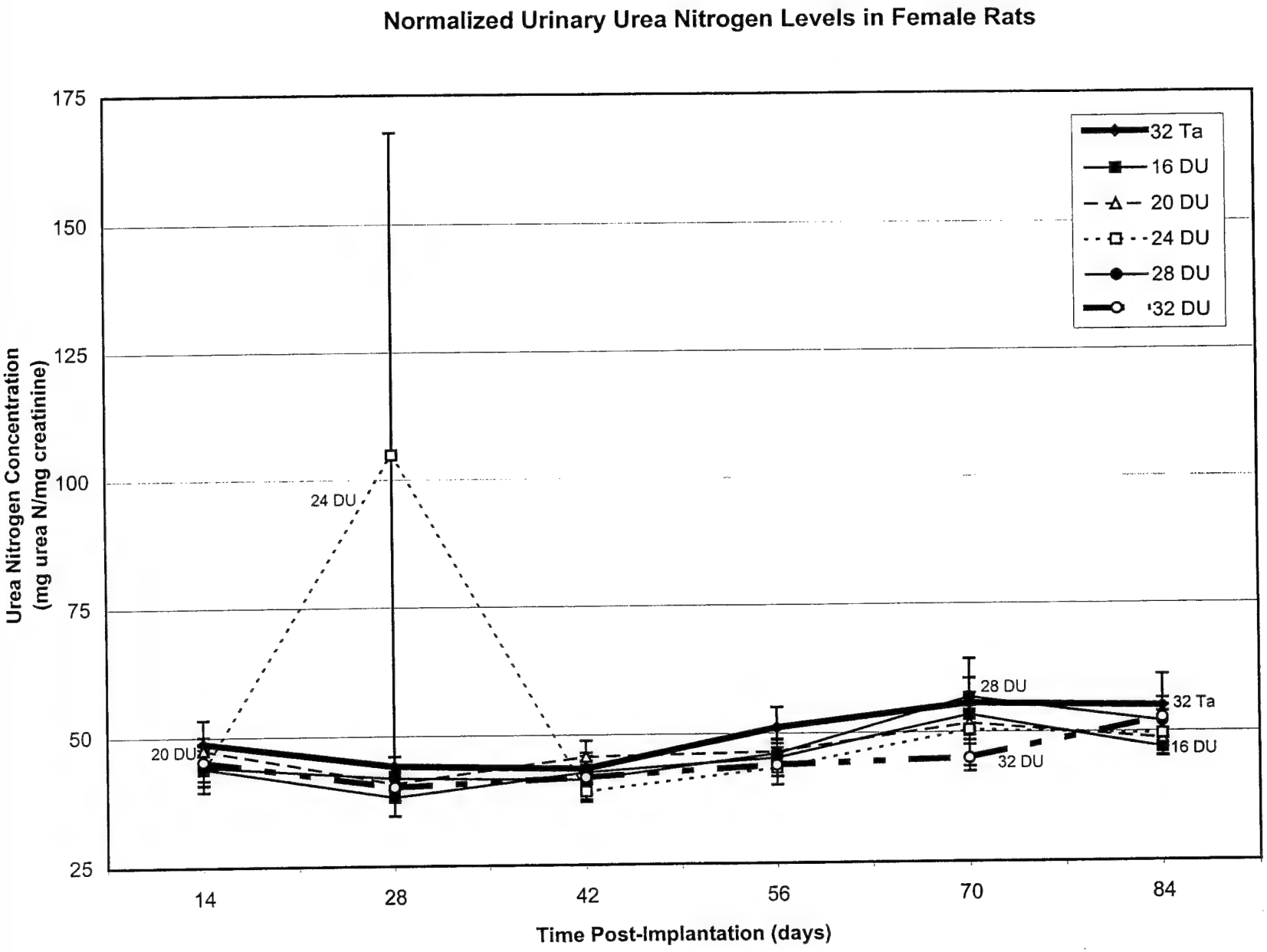

Normalized Urinary Urea Nitrogen Levels in Female Rats
Urea Nitrogen Concentration (mg urea N/mg creatinine)
175
150
125
100
75
50
25
14
28
42
56
70
84
Time Post-Implantation (days)
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
24 DU
20 DU
28 DU
32 Ta
16 DU
32 DU

Figure 8

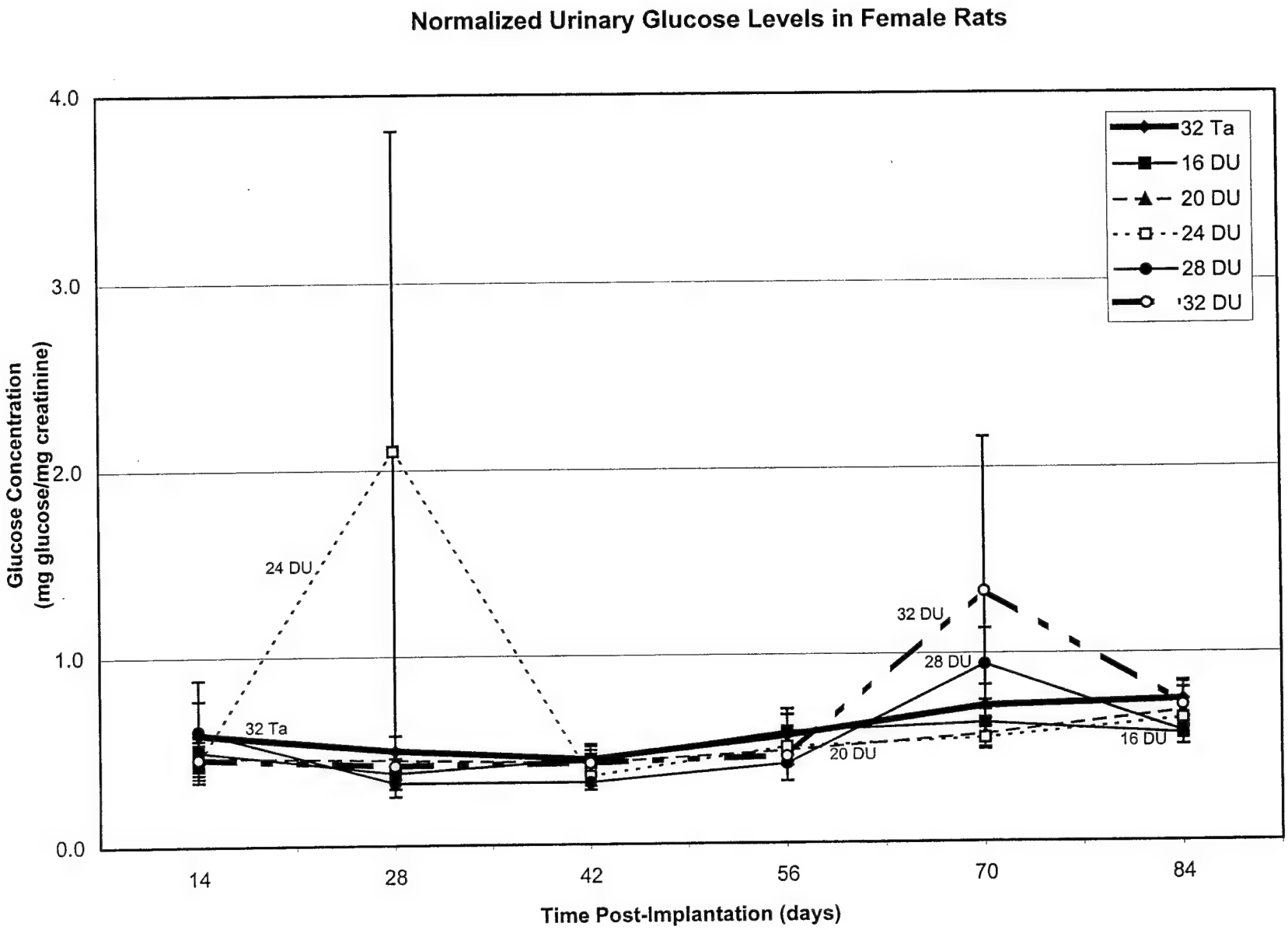

Normalized Urinary Glucose Levels in Female Rats
4.0
3.0
2.0
1.0
0.0
Glucose Concentration
(mg glucose/mg creatinine)
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
24 DU
32 DU
28 DU
32 Ta
20 DU
16 DU
14
28
42
56
70
84
Time Post-Implantation (days)

Figure 9

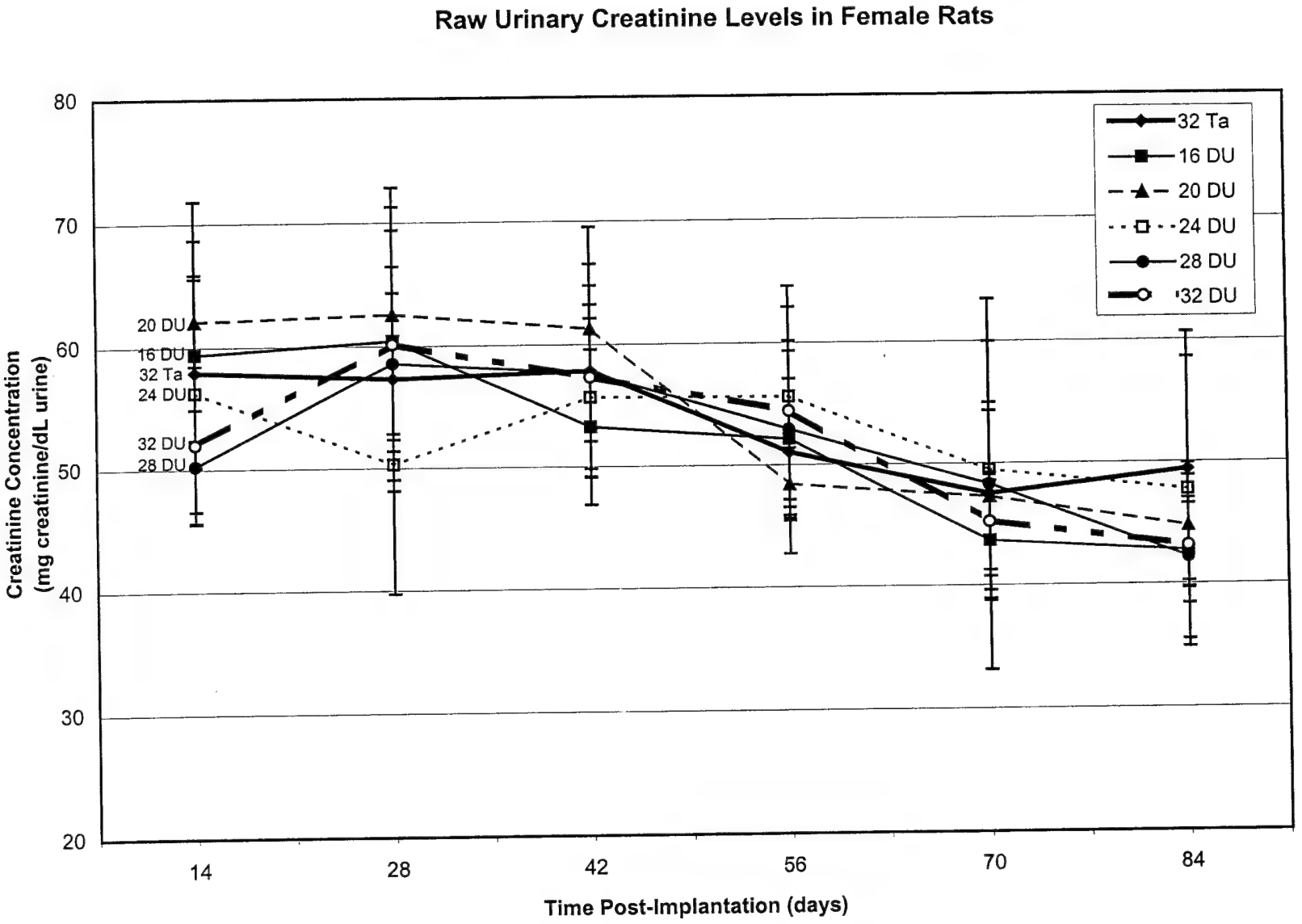

Raw Urinary Creatinine Levels in Female Rats
Creatinine Concentration (mg creatinine/dL urine)
80
70
60
50
40
30
20
14
28
42
56
70
84
Time Post-Implantation (days)
32 Ta
16 DU
20 DU
24 DU
28 DU
'32 DU
20 DU
16 DU
32 Ta
24 DU
32 DU
28 DU

Figure 10

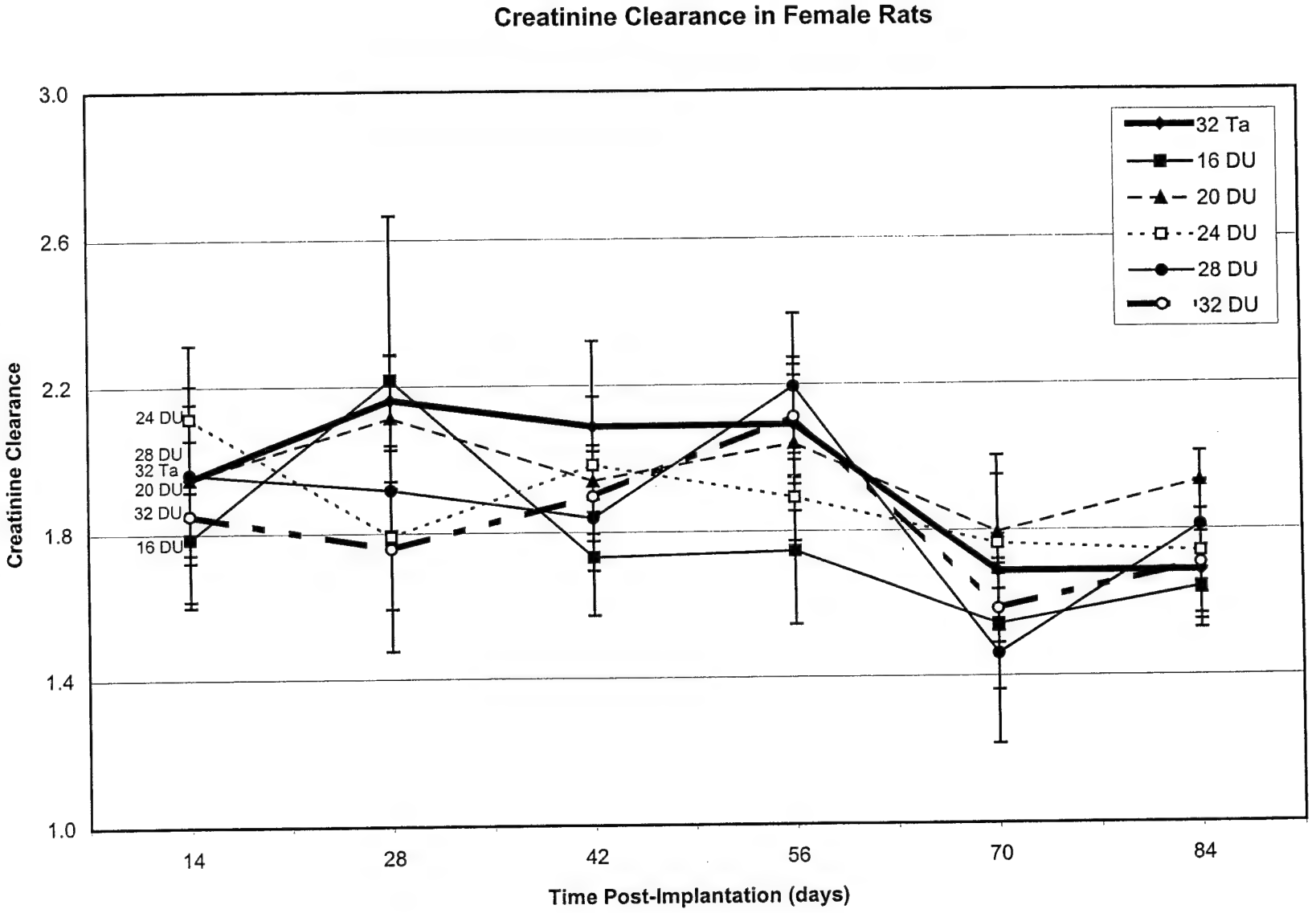

Creatinine Clearance in Female Rats
3.0
2.6
2.2
1.8
1.4
1.0
Creatinine Clearance
14
28
42
56
70
84
Time Post-Implantation (days)
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
24 DU
28 DU
32 Ta
20 DU
32 DU
16 DU

Figure 11

LDH in Urine Following DU Pellet Implantation

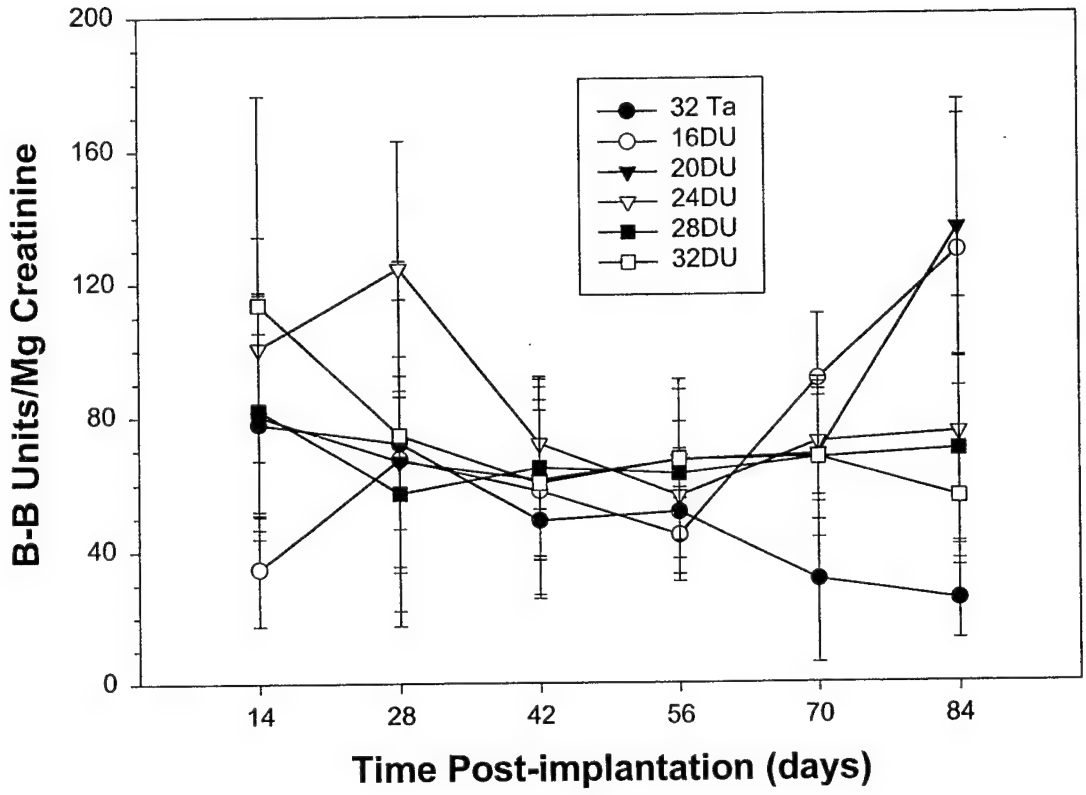


Figure 12

NAG in Urine Following DU Pellet Implantation

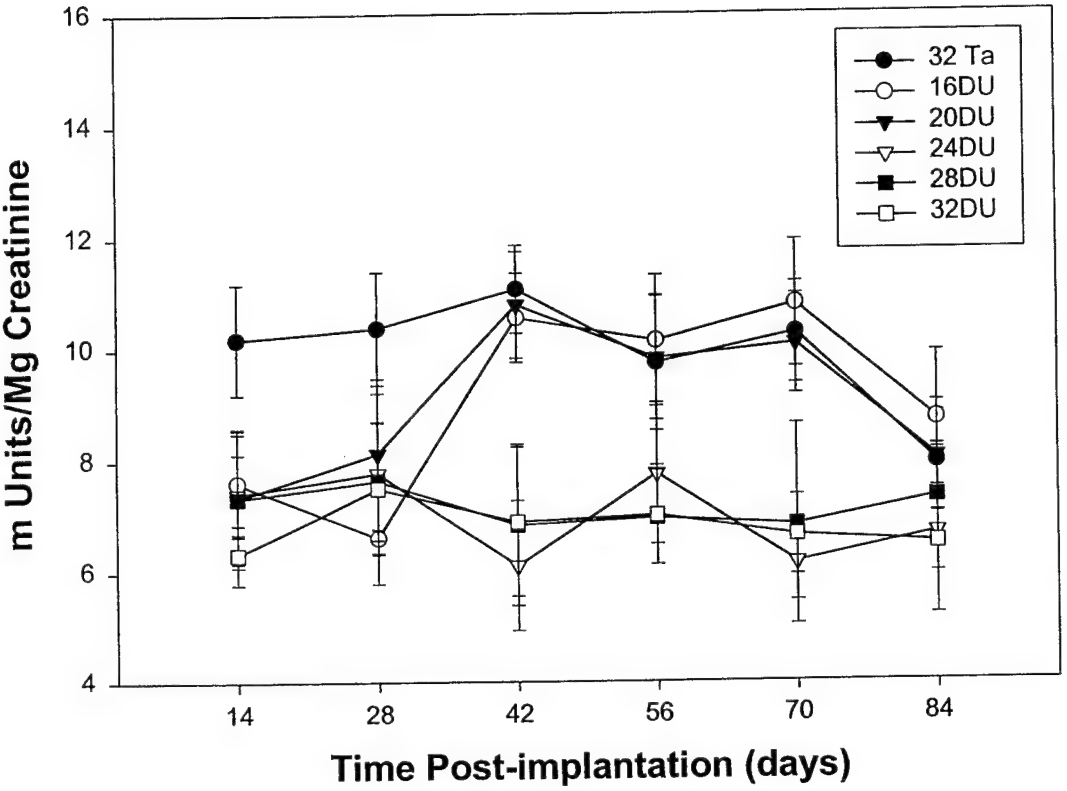


Figure 13

Osmolarity of Urine Following DU Pellet Implantation

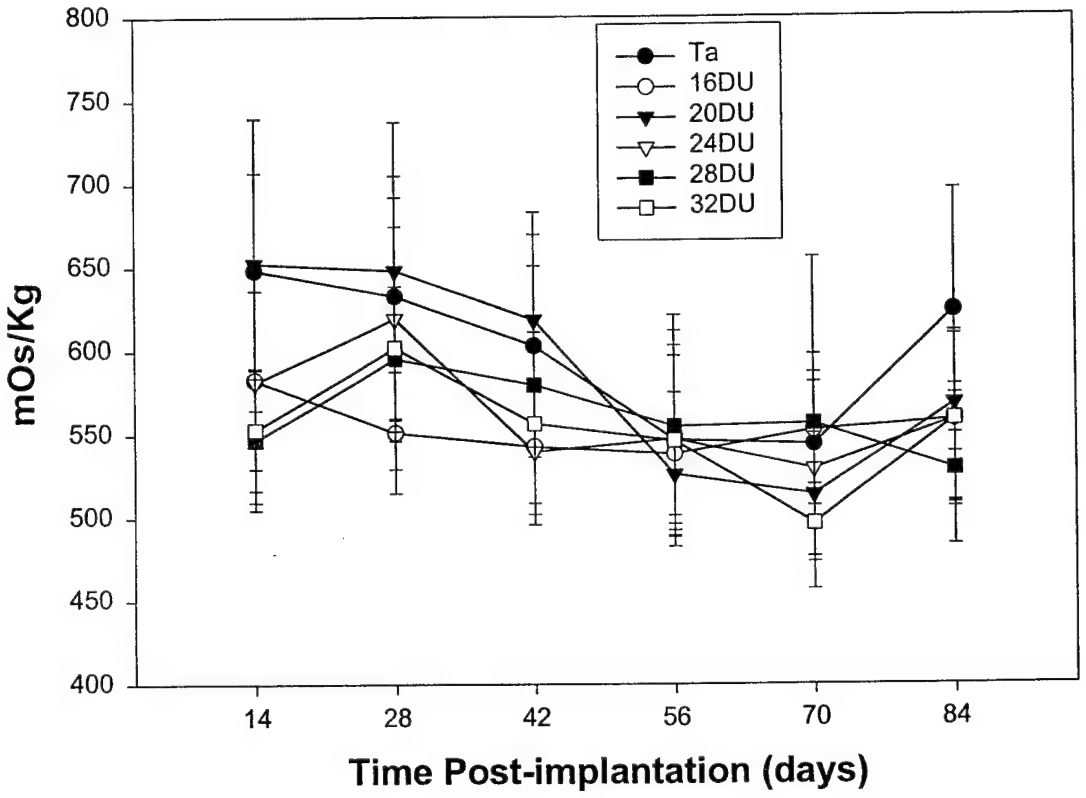


Figure 14

pH of Urine Following DU Pellet Implantation

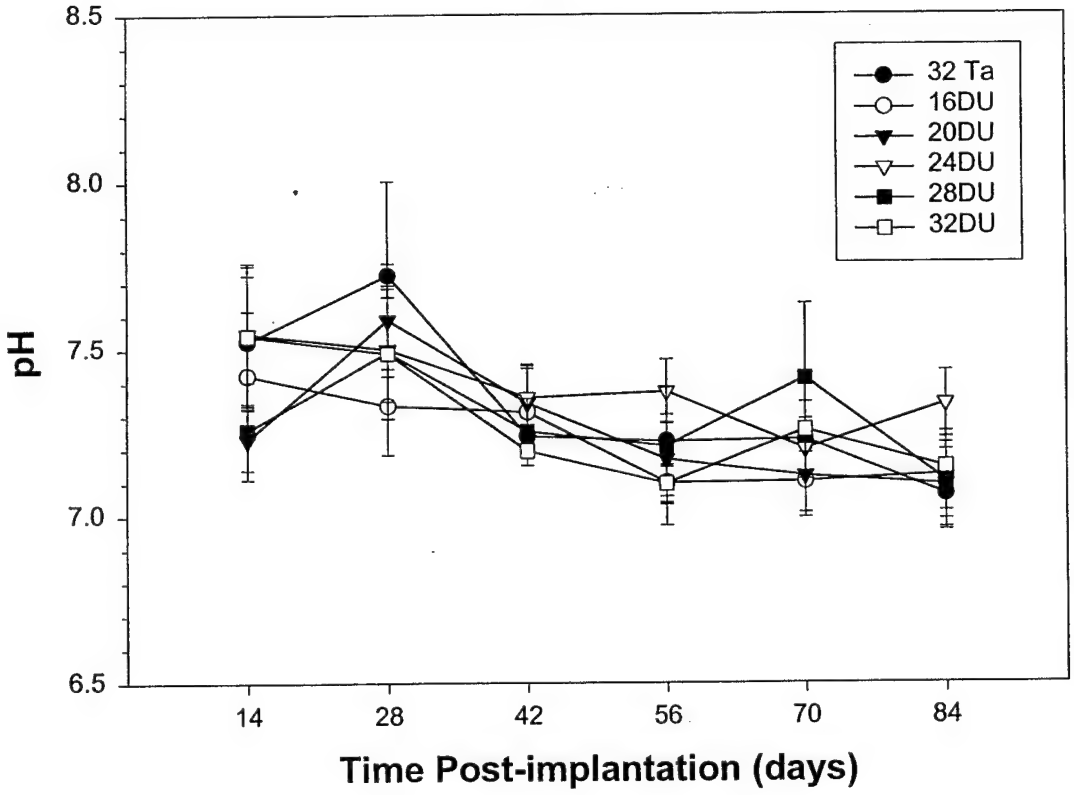


Figure 15

Protein in Urine Following DU Pellet Implantation

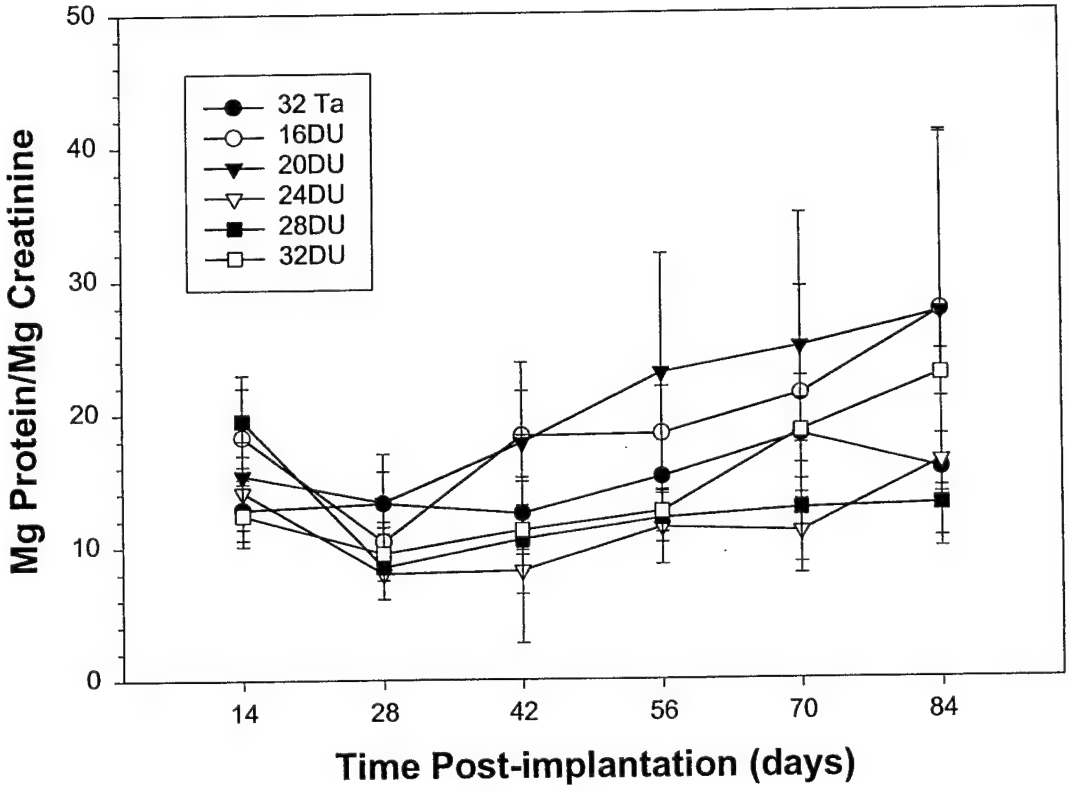


Figure 16

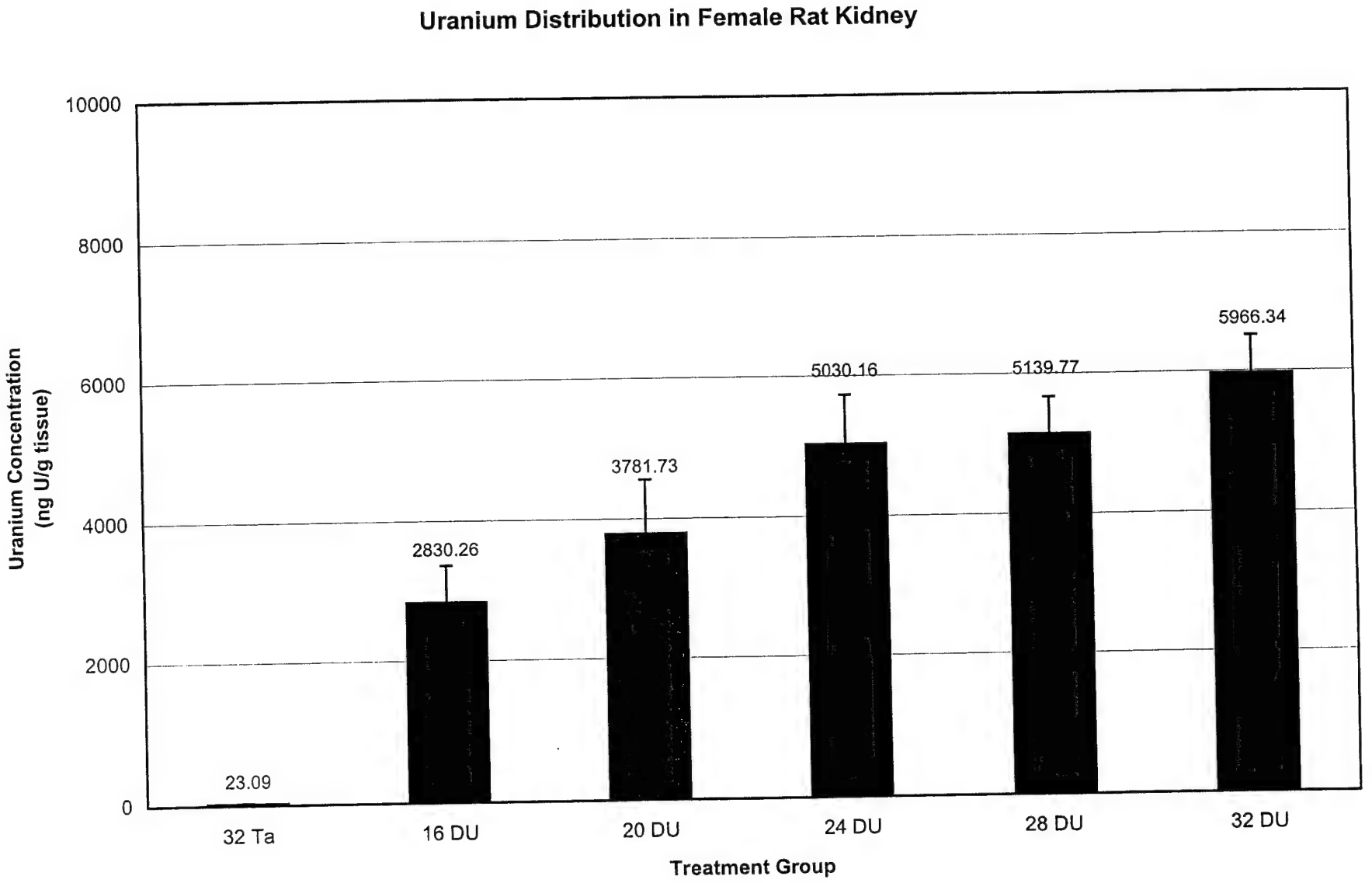

Uranium Distribution in Female Rat Kidney
Uranium Concentration (ng U/g tissue)
10000
8000
6000
4000
2000
0
23.09
2830.26
3781.73
5030.16
5139.77
5966.34
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 17

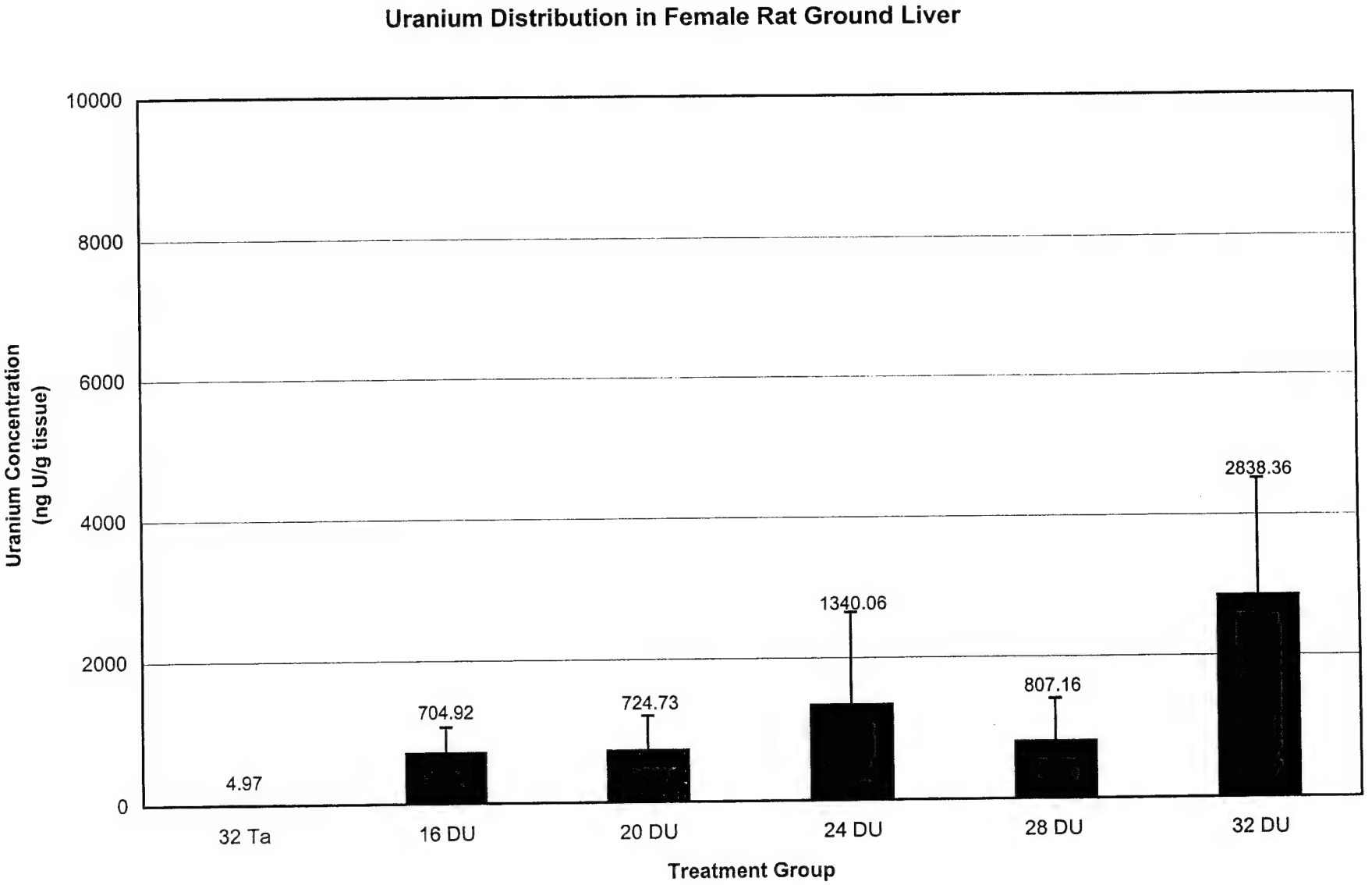

Uranium Distribution in Female Rat Ground Liver
Uranium Concentration (ng U/g tissue)
10000
8000
6000
4000
2000
0
4.97
704.92
724.73
1340.06
807.16
2838.36
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 18

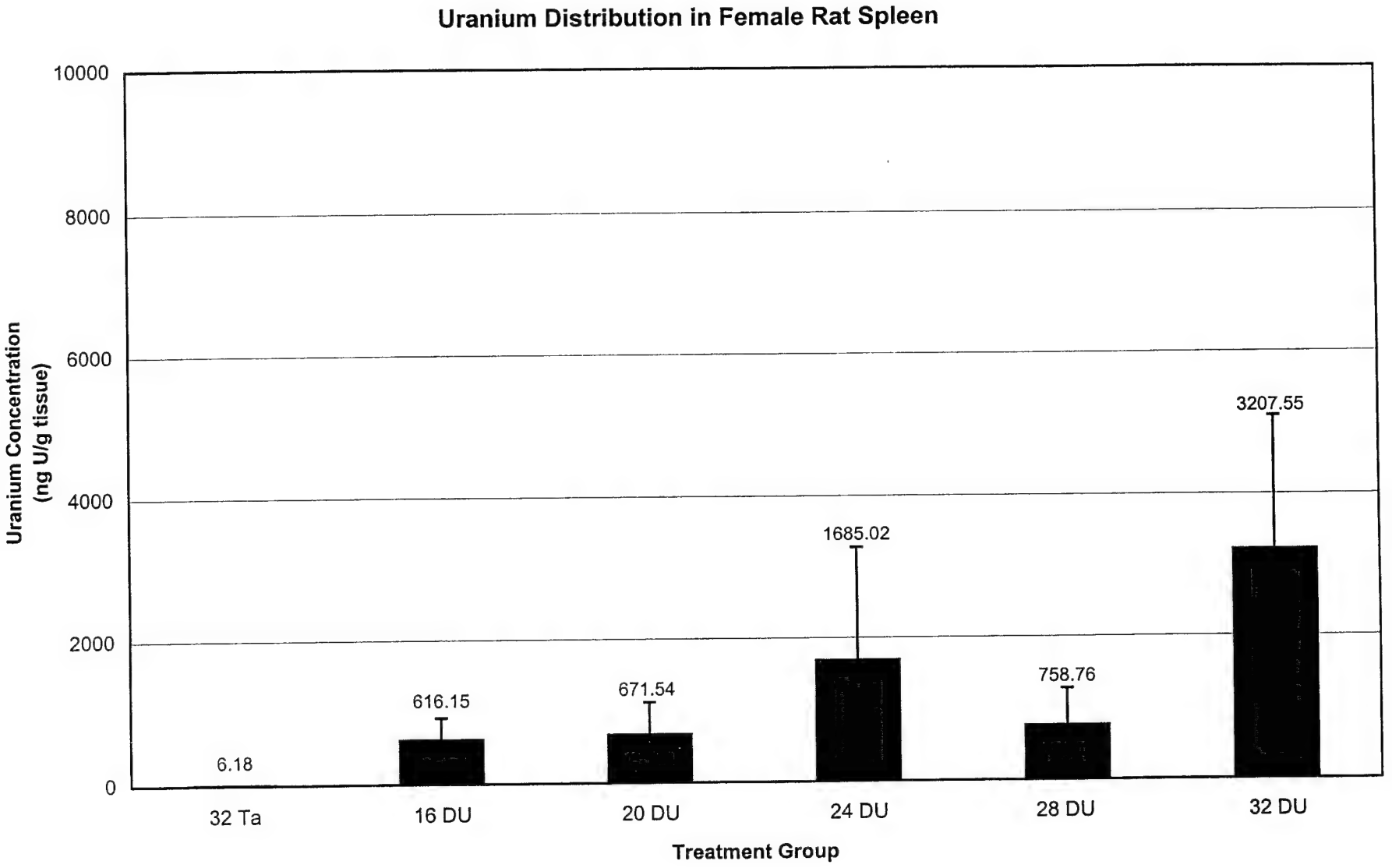

Uranium Distribution in Female Rat Spleen
Uranium Concentration (ng U/g tissue)
10000
8000
6000
4000
2000
0
6.18
616.15
671.54
1685.02
758.76
3207.55
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 19

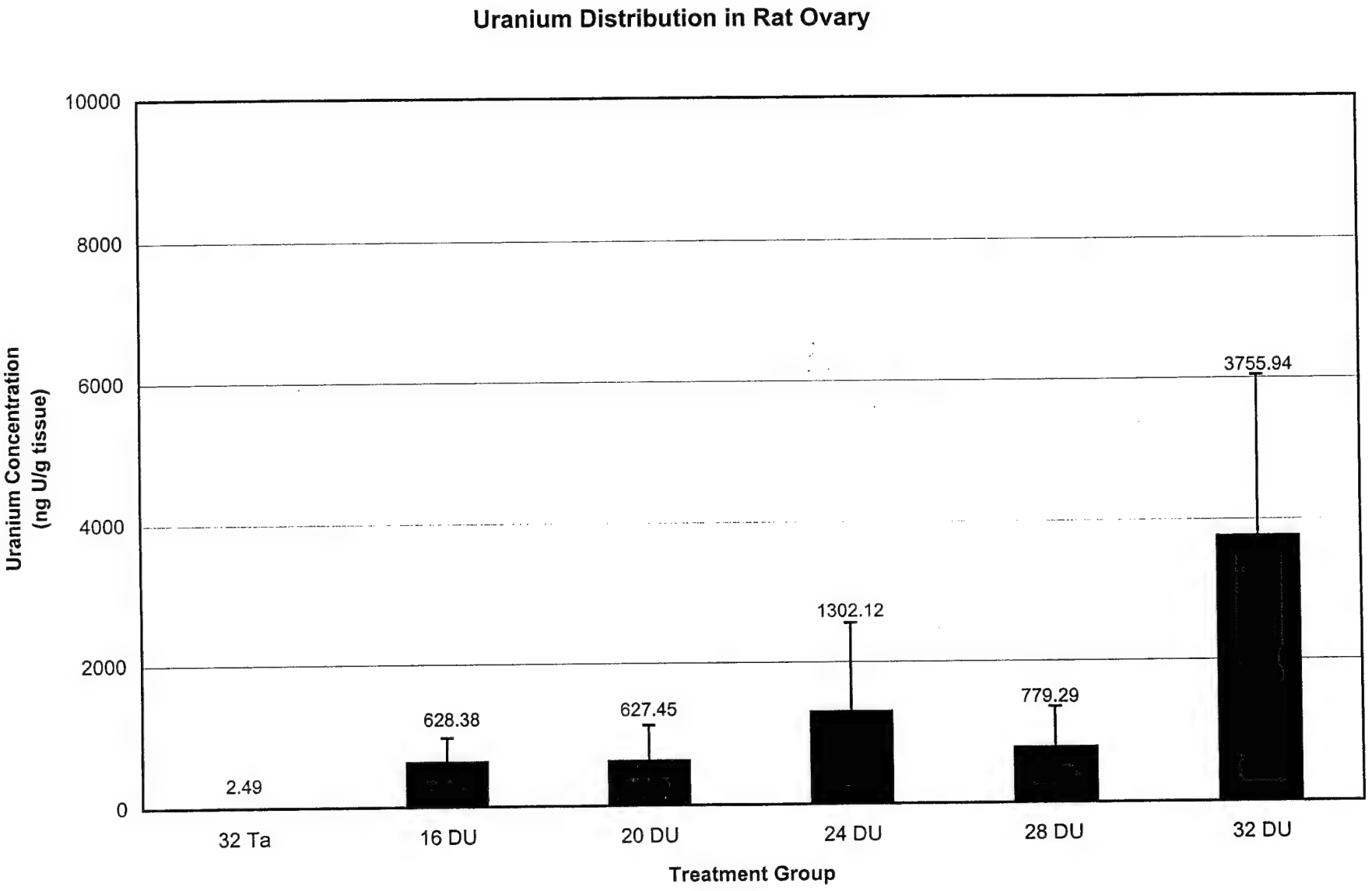

Uranium Distribution in Rat Ovary
Uranium Concentration (ng U/g tissue)
10000
8000
6000
4000
2000
0
2.49
628.38
627.45
1302.12
779.29
3755.94
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 20

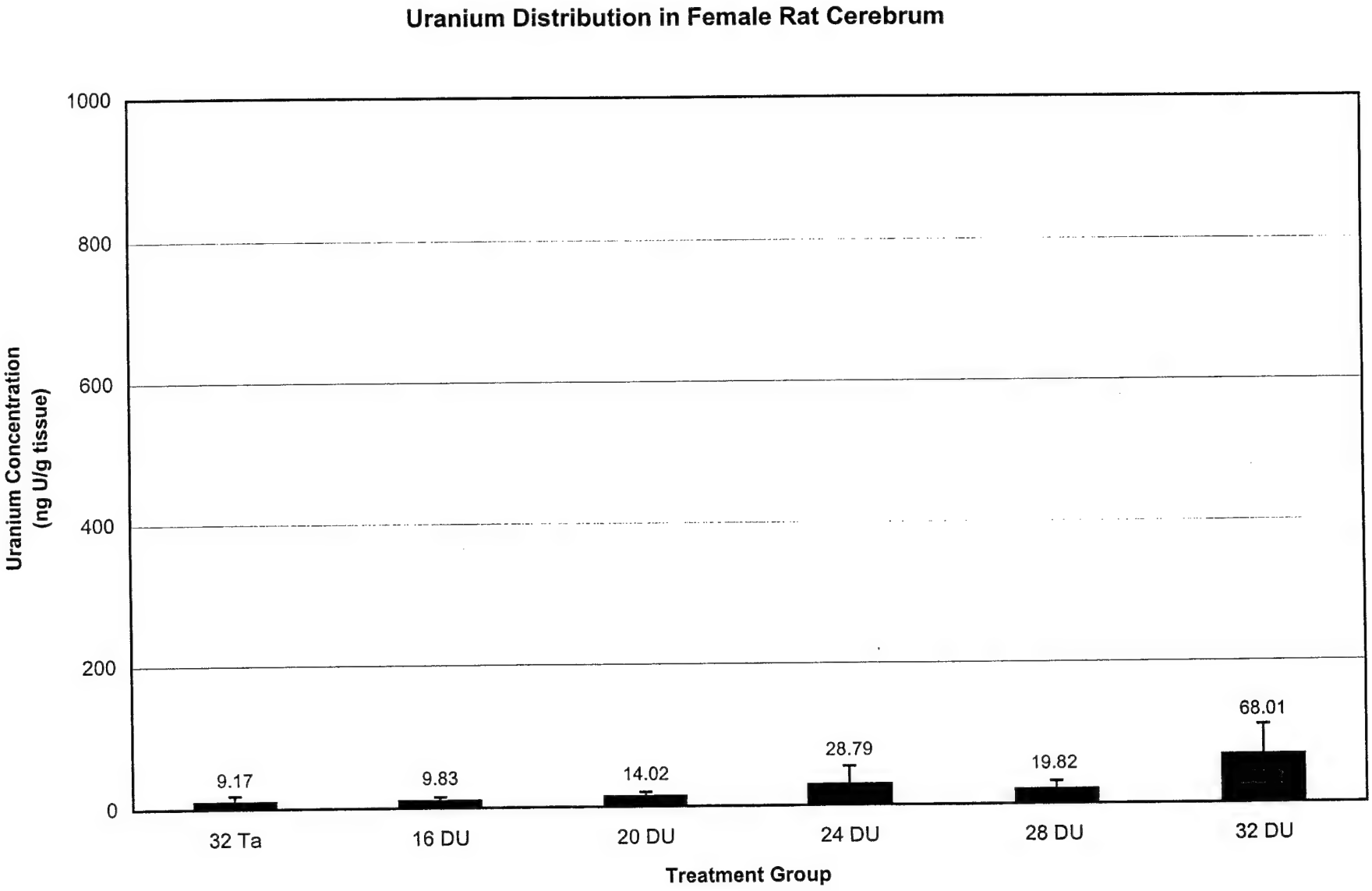

Uranium Distribution in Female Rat Cerebrum
Uranium Concentration (ng U/g tissue)
1000
800
600
400
200
0
9.17
9.83
14.02
28.79
19.82
68.01
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 21

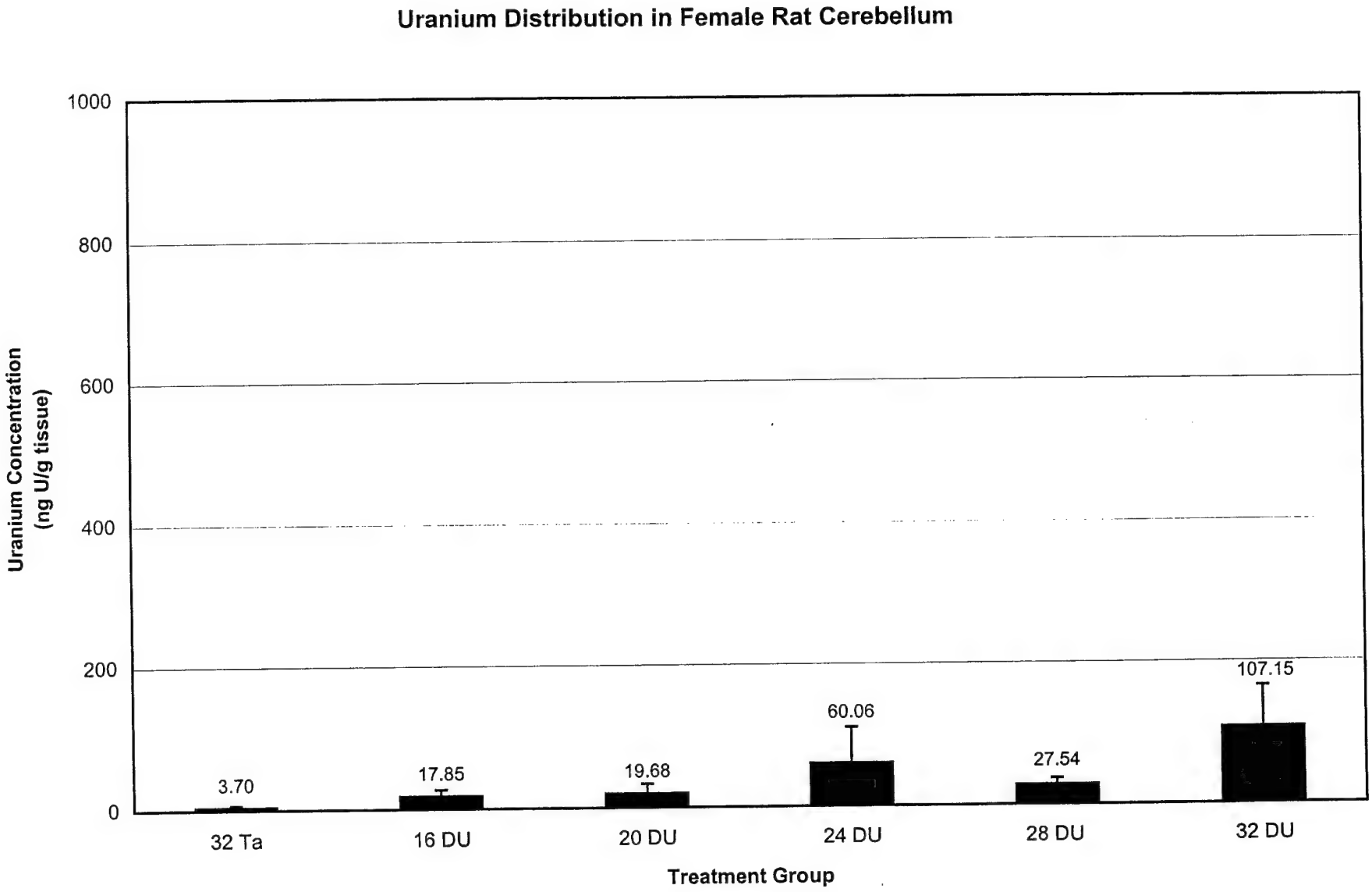

Uranium Distribution in Female Rat Cerebellum
Uranium Concentration (ng U/g tissue)
1000
800
600
400
200
0
3.70
17.85
19.68
60.06
27.54
107.15
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 22

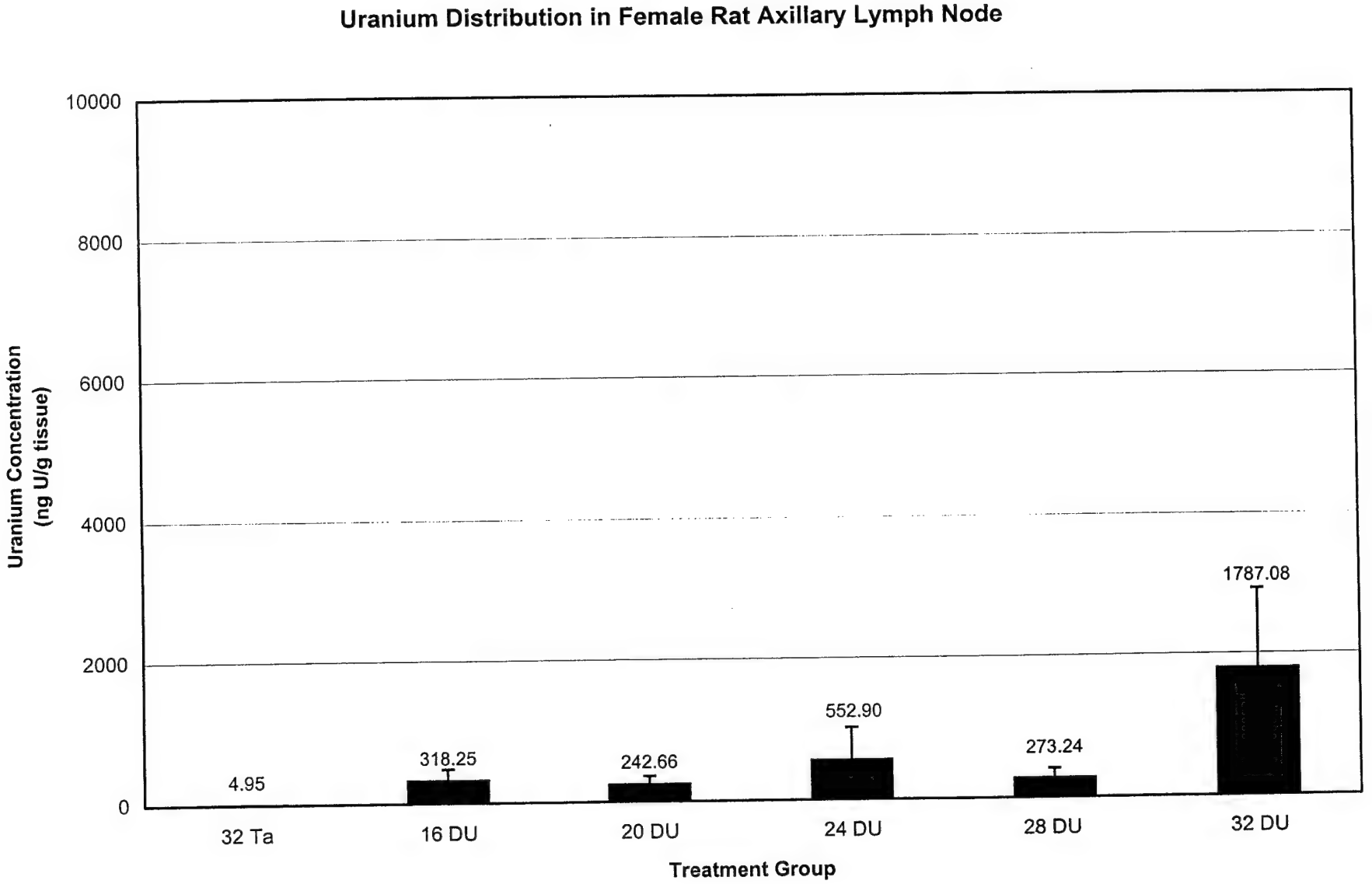

Uranium Distribution in Female Rat Axillary Lymph Node
Uranium Concentration (ng U/g tissue)
10000
8000
6000
4000
2000
0
4.95
318.25
242.66
552.90
273.24
1787.08
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 23

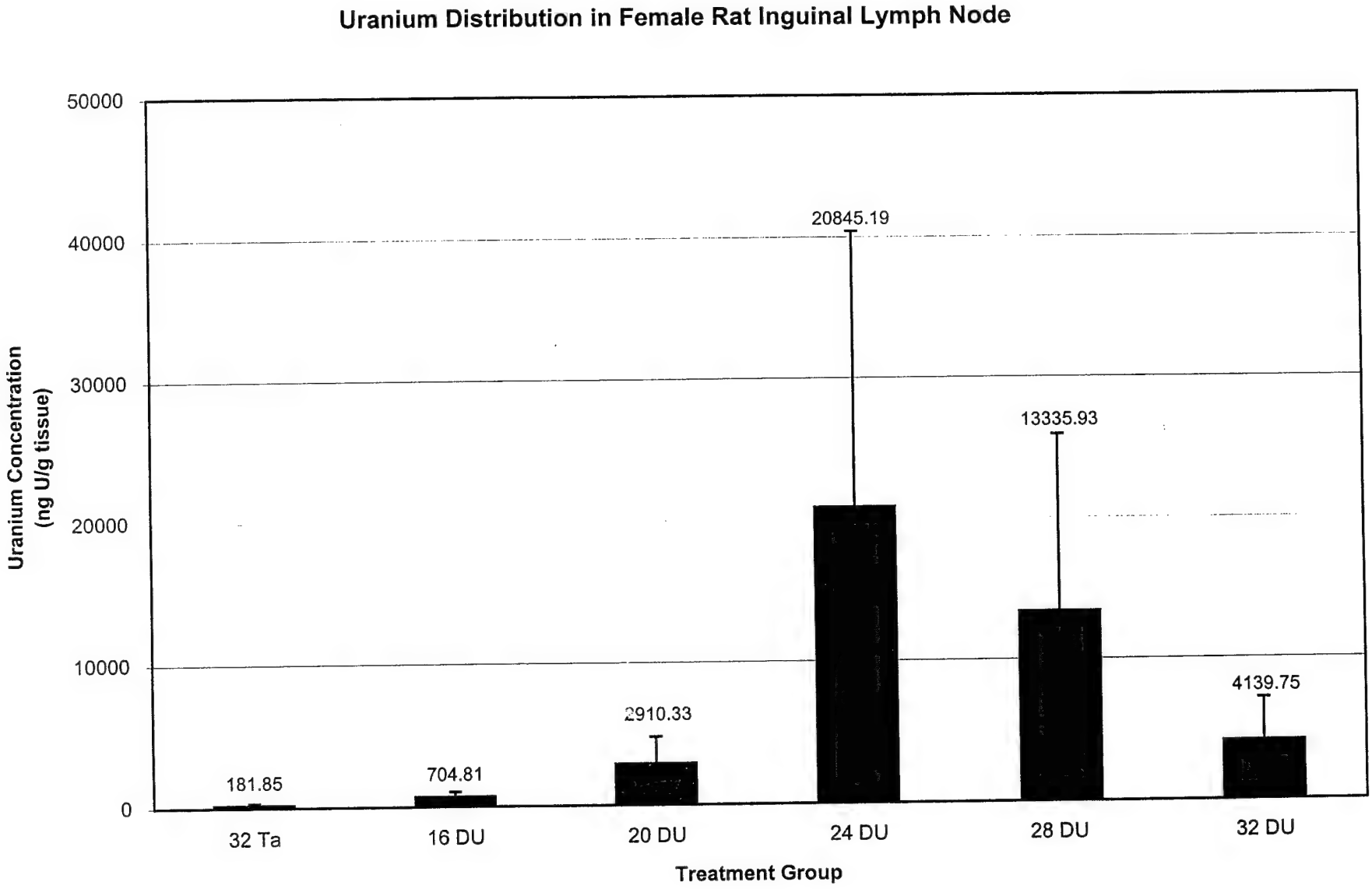

Uranium Distribution in Female Rat Inguinal Lymph Node
Uranium Concentration (ng U/g tissue)
50000
40000
30000
20000
10000
0
181.85
704.81
2910.33
20845.19
13335.93
4139.75
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 24

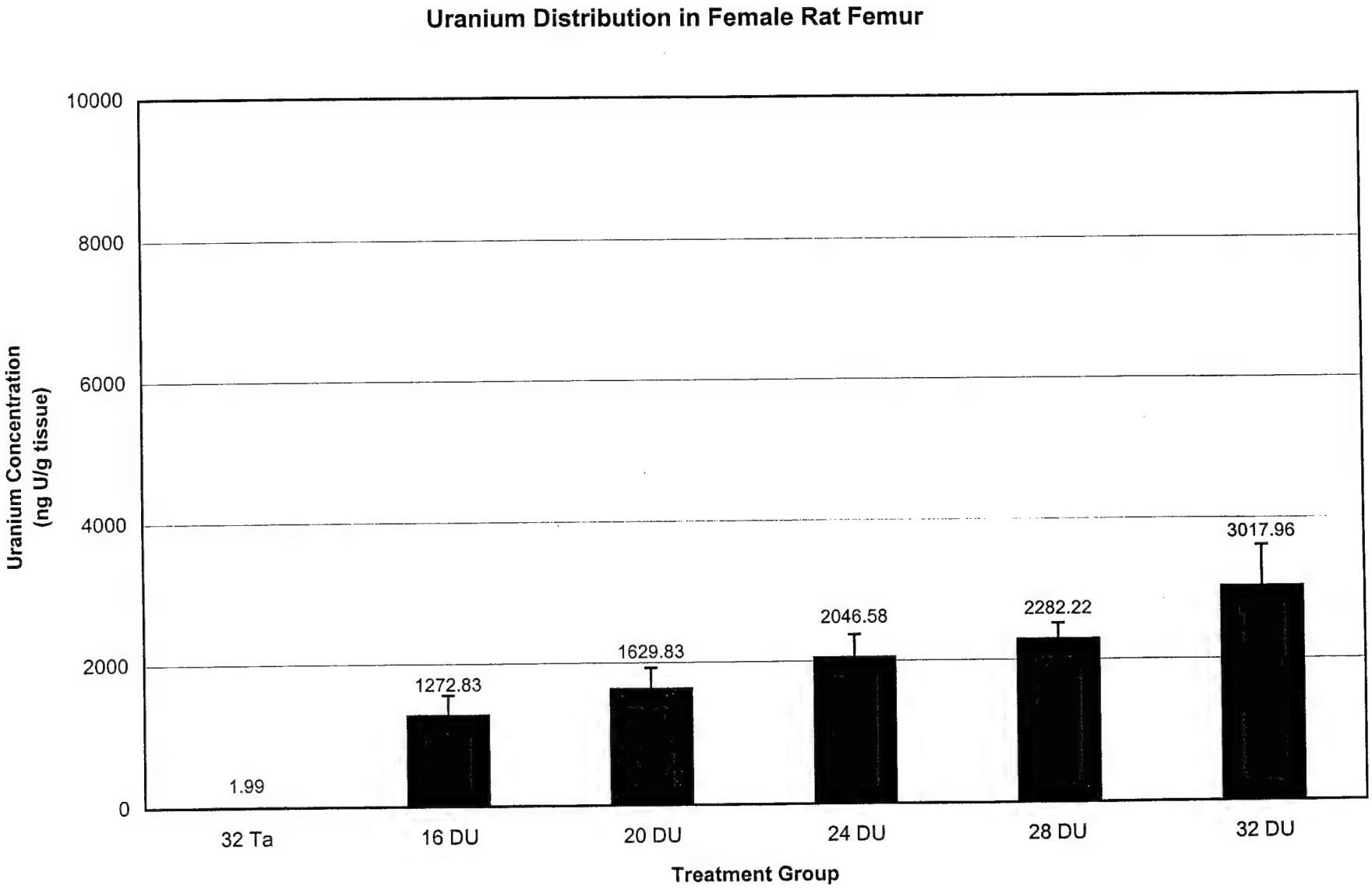

Uranium Distribution in Female Rat Femur
Uranium Concentration (ng U/g tissue)
10000
8000
6000
4000
2000
0
1.99
1272.83
1629.83
2046.58
2282.22
3017.96
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 25

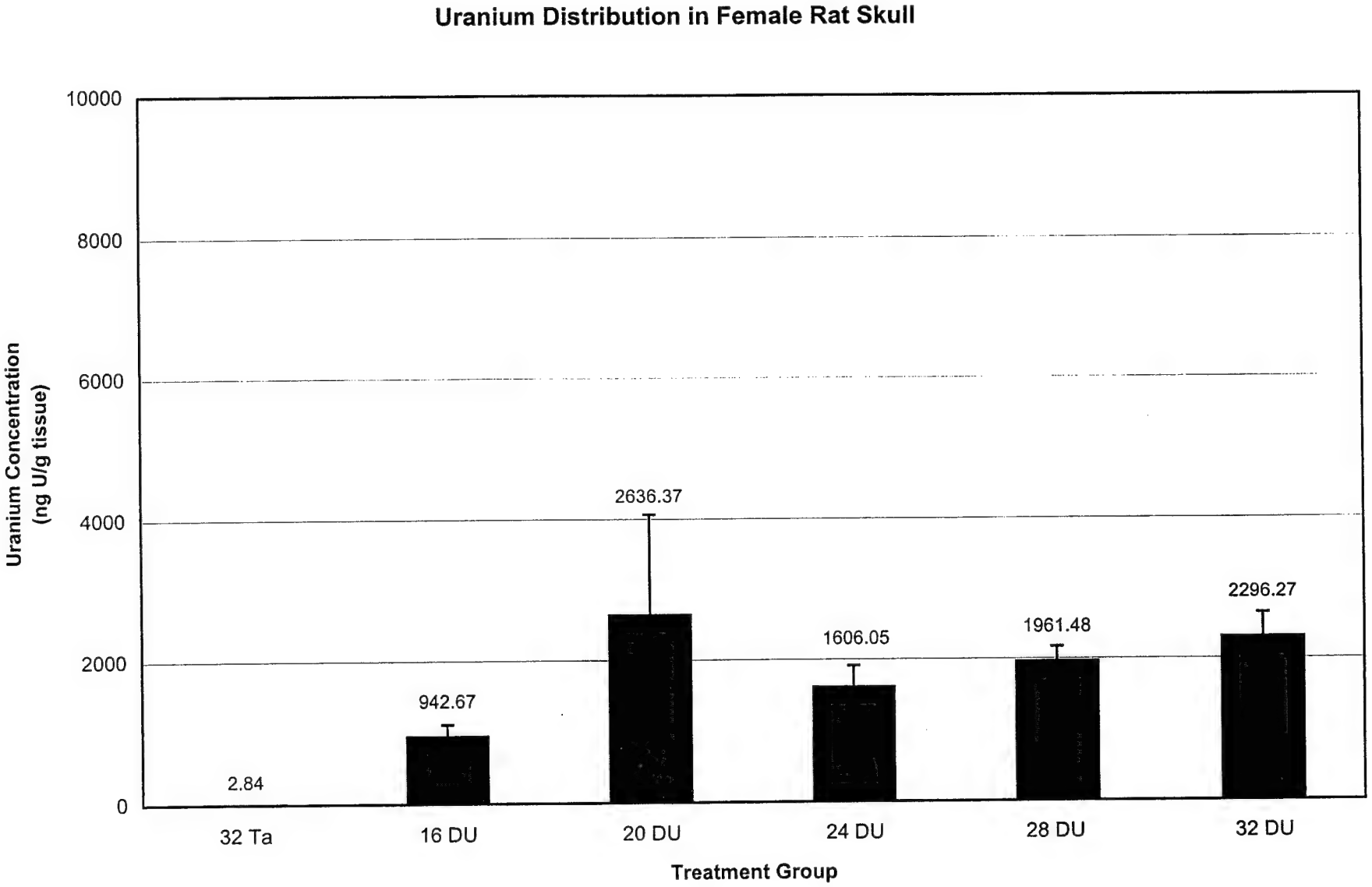

Uranium Distribution in Female Rat Skull
10000
8000
6000
4000
2000
0
Uranium Concentration (ng U/g tissue)
2.84
942.67
2636.37
1606.05
1961.48
2296.27
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 26

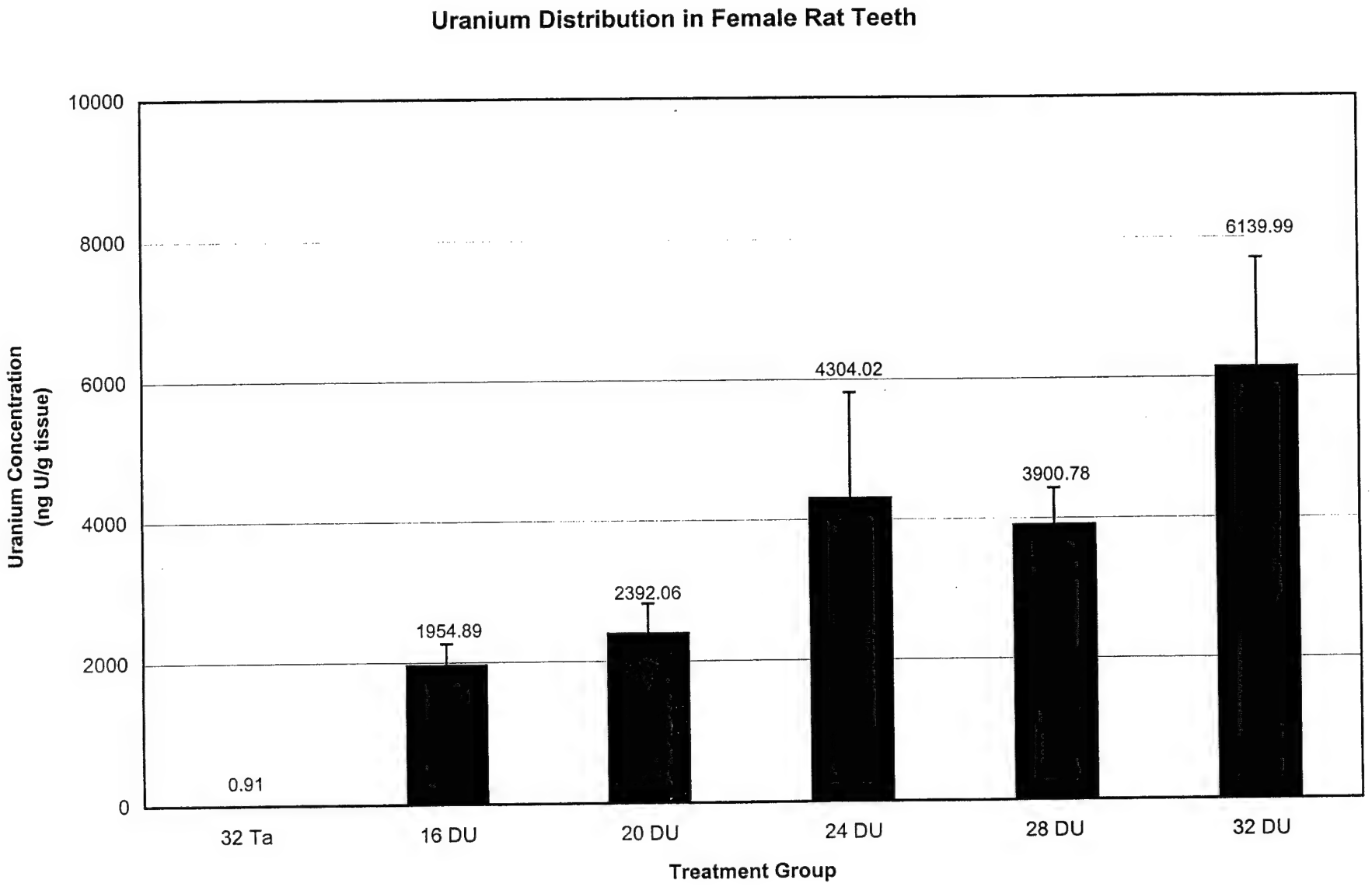

Uranium Distribution in Female Rat Teeth
Uranium Concentration (ng U/g tissue)
10000
8000
6000
4000
2000
0
0.91
1954.89
2392.06
4304.02
3900.78
6139.99
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 27

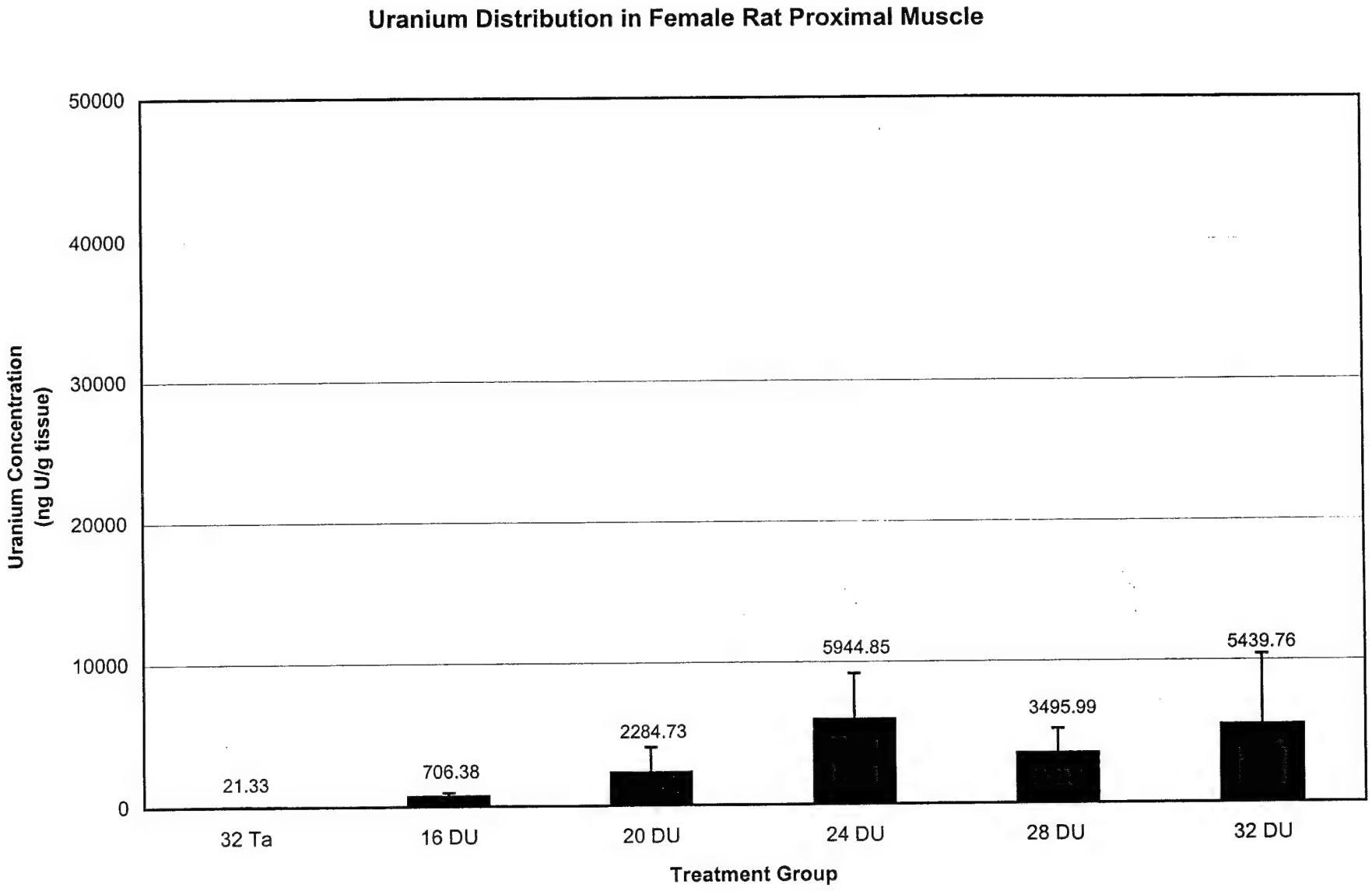

Uranium Distribution in Female Rat Proximal Muscle
Uranium Concentration (ng U/g tissue)
50000
40000
30000
20000
10000
0
21.33
706.38
2284.73
5944.85
3495.99
5439.76
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 28

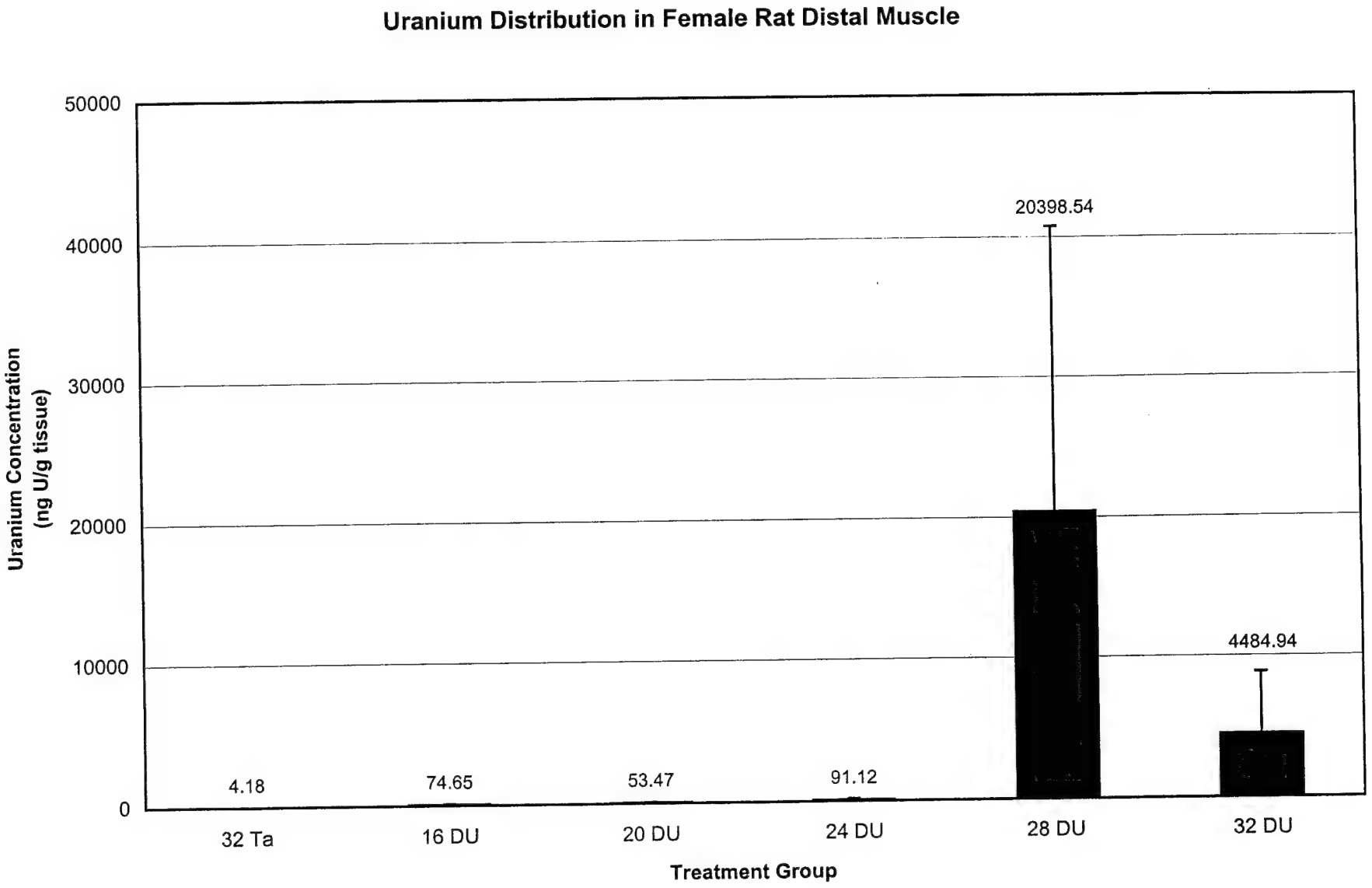

Uranium Distribution in Female Rat Distal Muscle
50000
40000
30000
20000
10000
0
Uranium Concentration (ng U/g tissue)
20398.54
4484.94
4.18
74.65
53.47
91.12
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 29

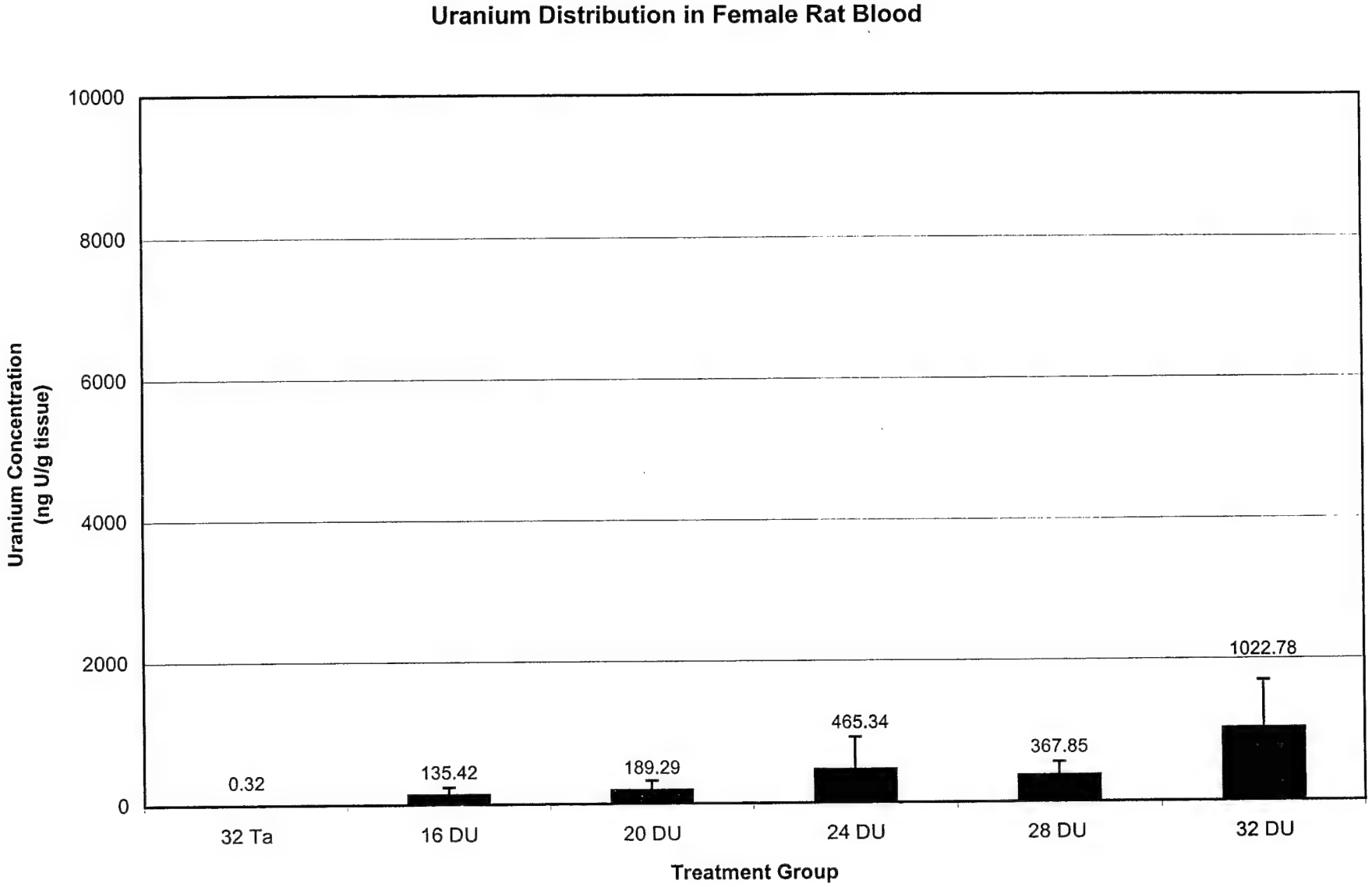

Uranium Distribution in Female Rat Blood
Uranium Concentration (ng U/g tissue)
10000
8000
6000
4000
2000
0
0.32
135.42
189.29
465.34
367.85
1022.78
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Treatment Group

Figure 30

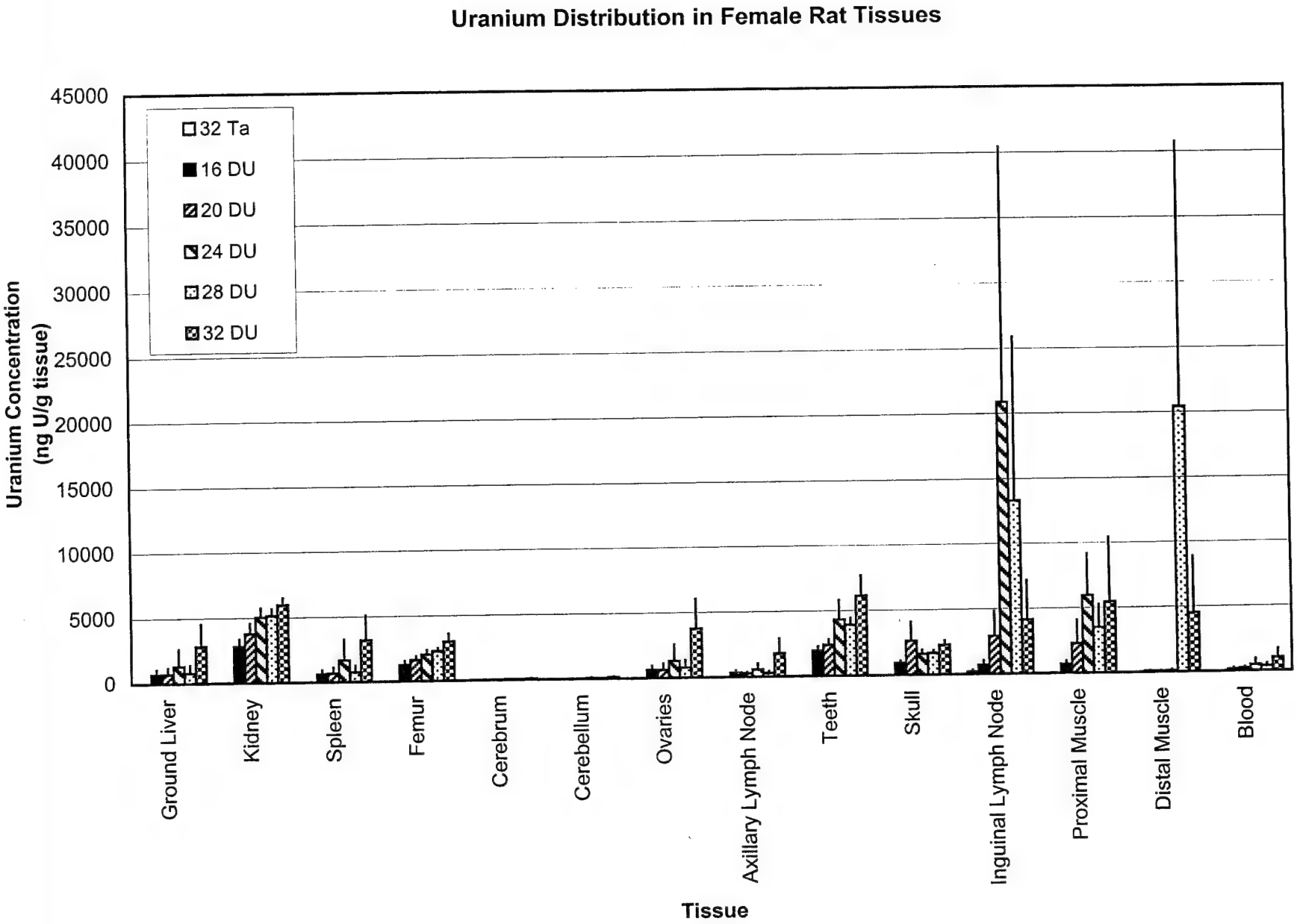

Uranium Distribution in Female Rat Tissues
Uranium Concentration (ng U/g tissue)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
Ground Liver
Kidney
Spleen
Femur
Cerebrum
Cerebellum
Ovaries
Axillary Lymph Node
Teeth
Skull
Inguinal Lymph Node
Proximal Muscle
Distal Muscle
Blood
Tissue

Figure 31

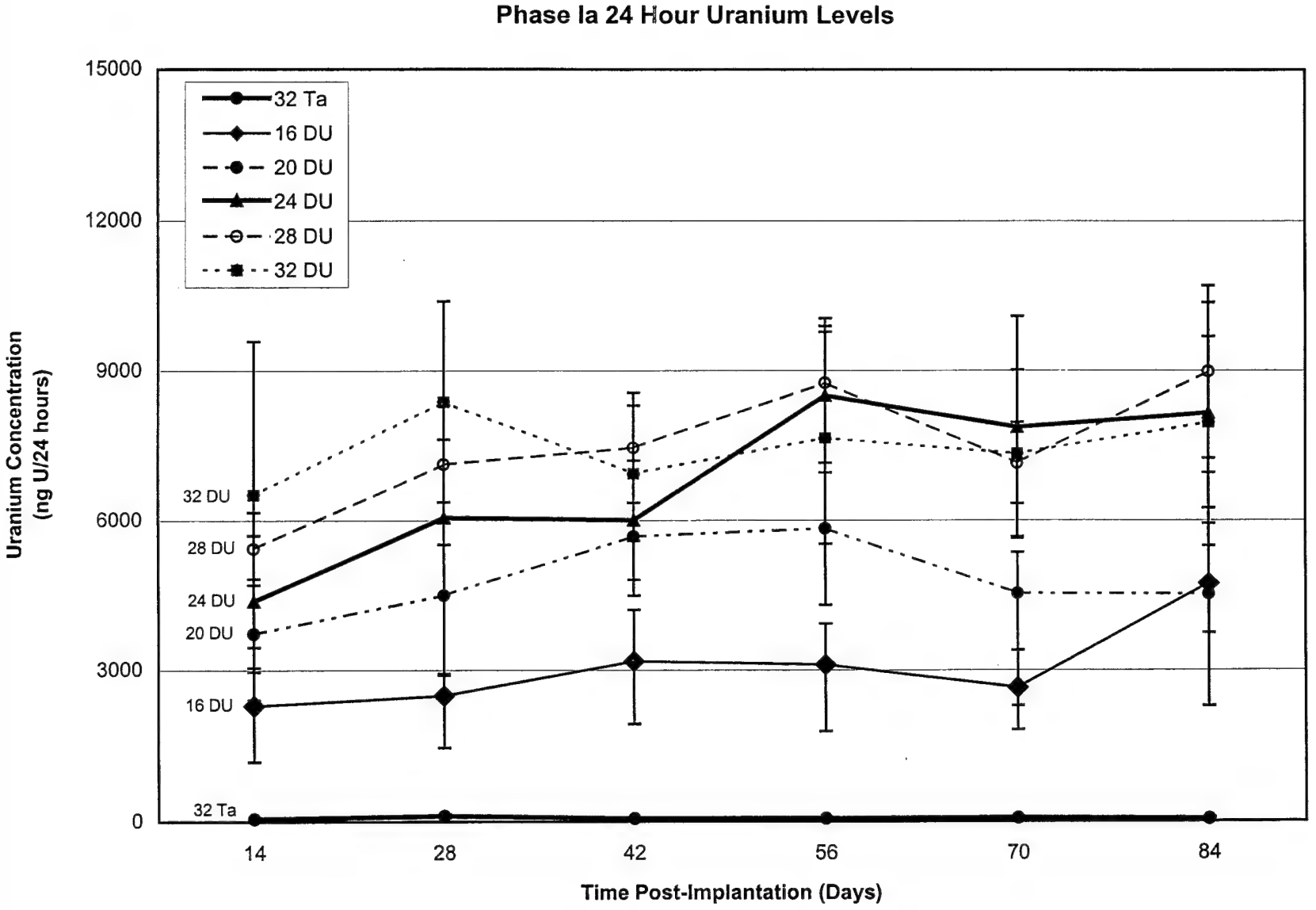

Phase Ia 24 Hour Uranium Levels
15000
12000
9000
6000
3000
0
32 Ta
16 DU
20 DU
24 DU
28 DU
32 DU
32 DU
28 DU
24 DU
20 DU
16 DU
32 Ta
14
28
42
56
70
84
Uranium Concentration (ng U/24 hours)
Time Post-Implantation (Days)

Figure 32

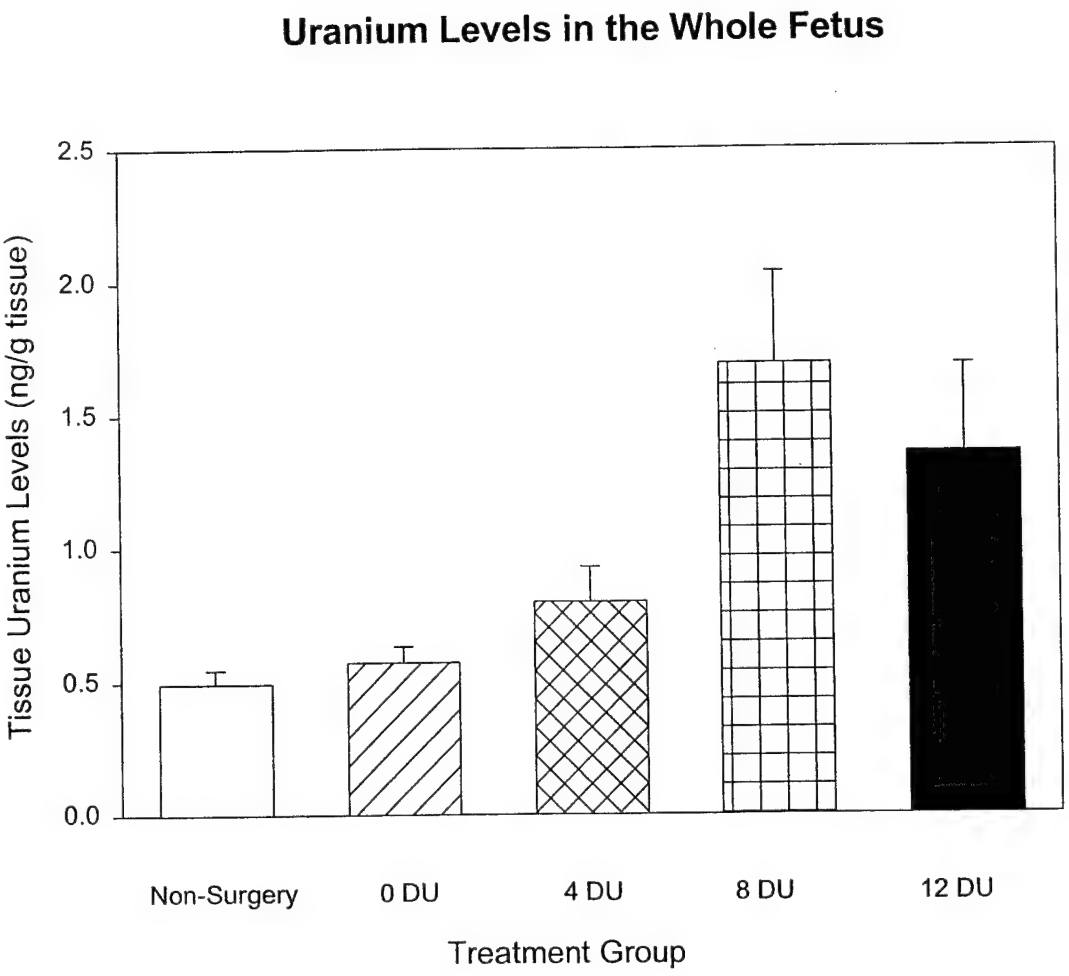

Uranium Levels in the Whole Fetus
Tissue Uranium Levels (ng/g tissue)
2.5
2.0
1.5
1.0
0.5
0.0
Non-Surgery
0 DU
4 DU
8 DU
12 DU
Treatment Group

Figure 33

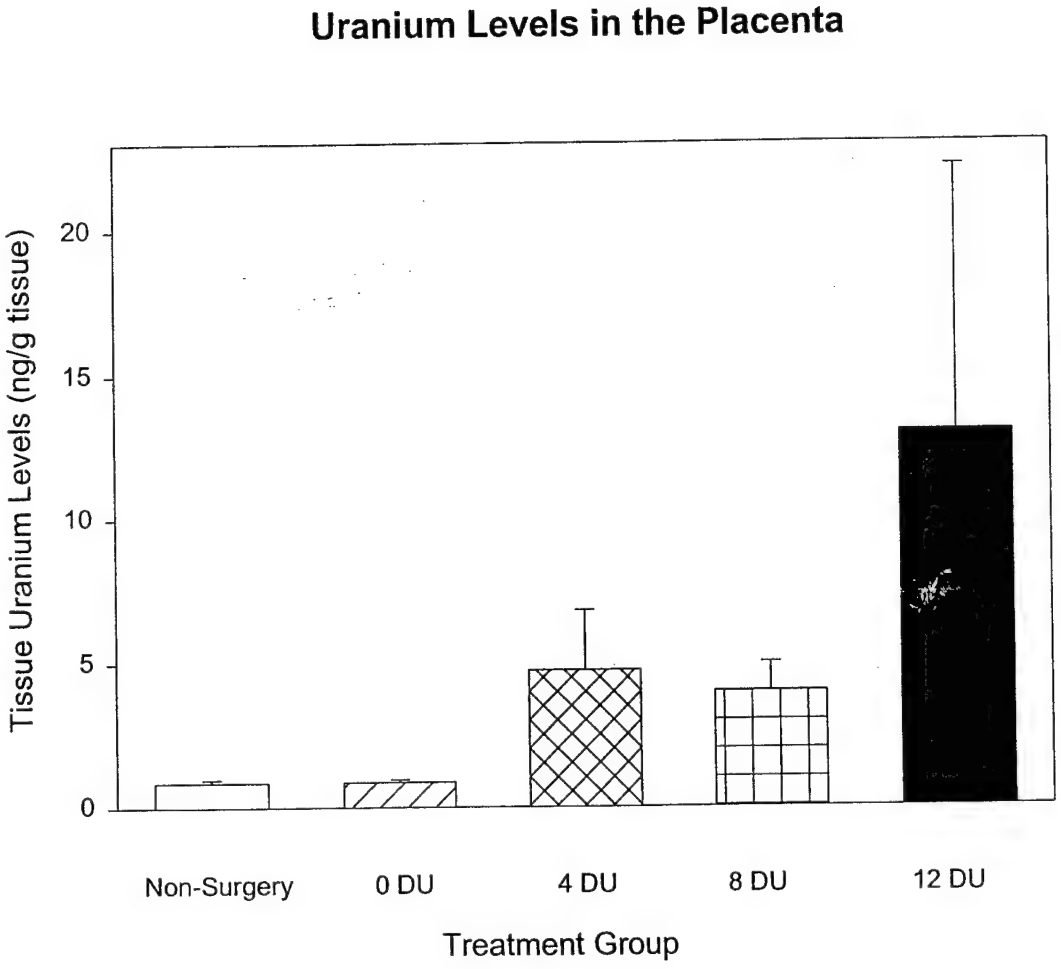

Uranium Levels in the Placenta
Tissue Uranium Levels (ng/g tissue)
0
5
10
15
20
Non-Surgery
0 DU
4 DU
8 DU
12 DU
Treatment Group

Figure 34

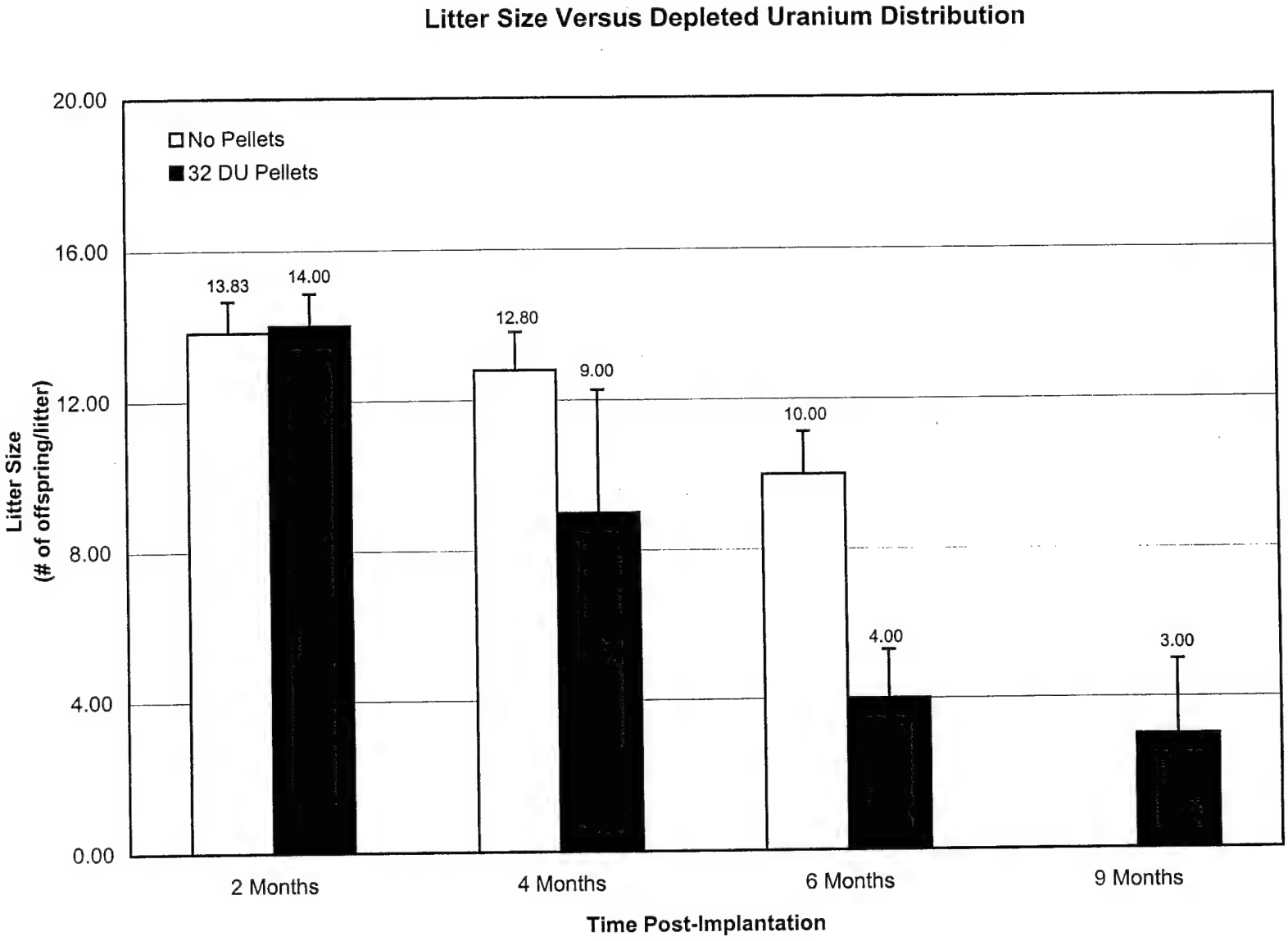

Litter Size Versus Depleted Uranium Distribution
20.00
16.00
12.00
8.00
4.00
0.00
No Pellets
32 DU Pellets
13.83
14.00
12.80
9.00
10.00
4.00
3.00
Litter Size
(# of offspring/litter)
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 35

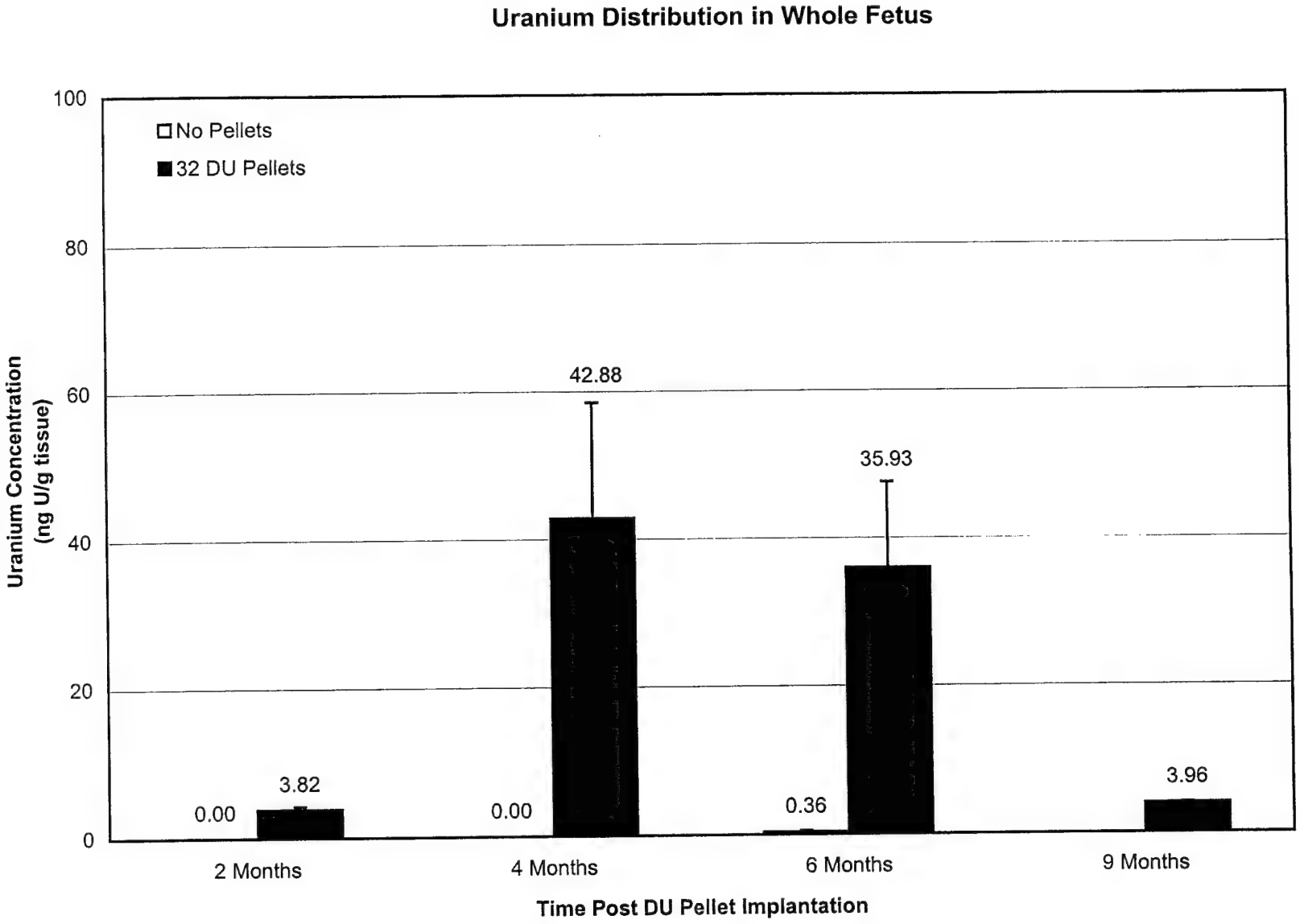

Uranium Distribution in Whole Fetus
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
100
80
60
40
20
0
0.00
3.82
0.00
42.88
0.36
35.93
3.96
2 Months
4 Months
6 Months
9 Months
Time Post DU Pellet Implantation

Figure 36

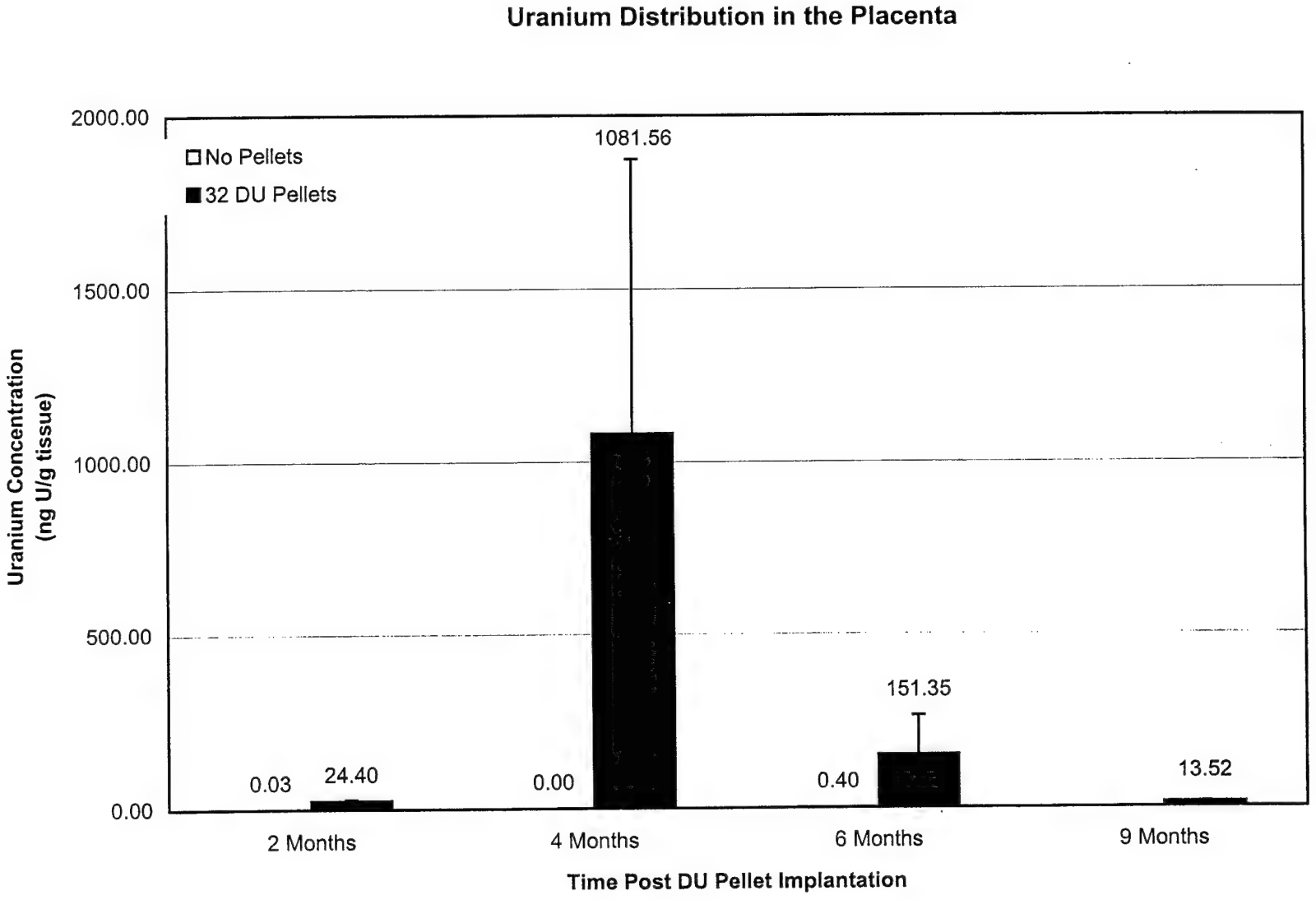

Uranium Distribution in the Placenta
2000.00
1500.00
1000.00
500.00
0.00
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
0.03
24.40
0.00
1081.56
0.40
151.35
13.52
2 Months
4 Months
6 Months
9 Months
Time Post DU Pellet Implantation

Figure 37

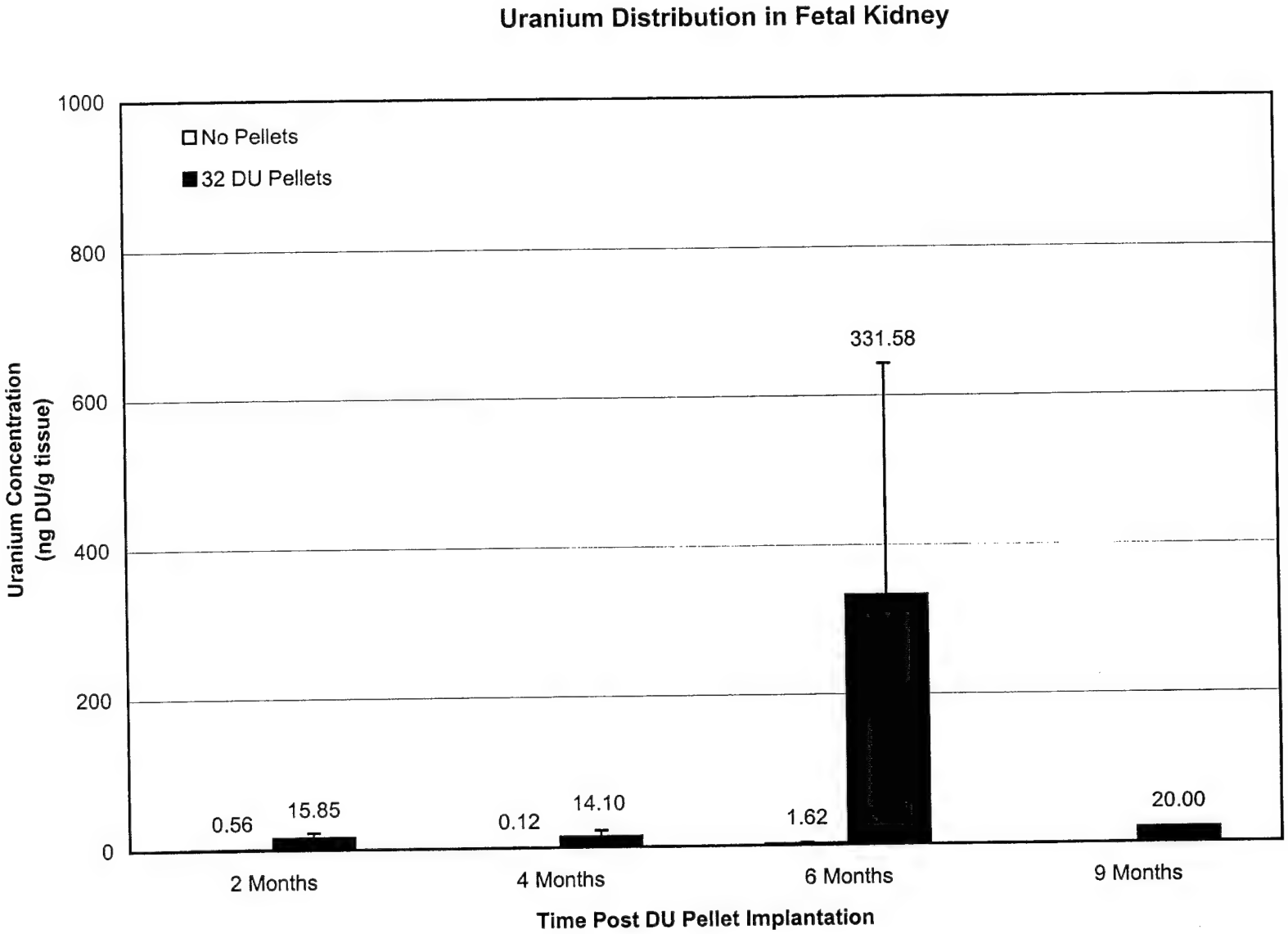

Uranium Distribution in Fetal Kidney
1000
800
600
400
200
0
No Pellets
32 DU Pellets
Uranium Concentration (ng DU/g tissue)
0.56
15.85
0.12
14.10
1.62
331.58
20.00
2 Months
4 Months
6 Months
9 Months
Time Post DU Pellet Implantation

Figure 38

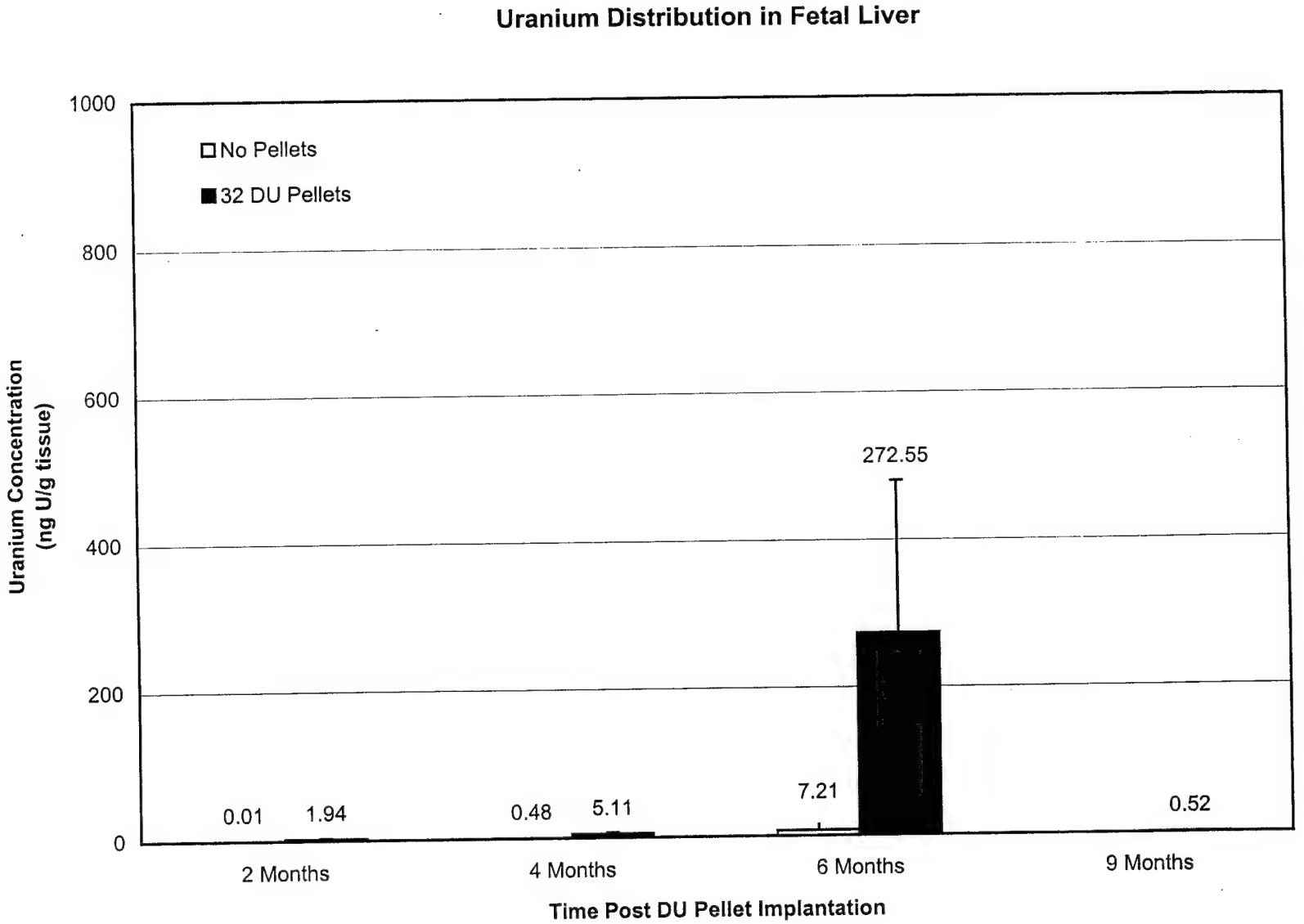

Uranium Distribution in Fetal Liver
1000
800
600
400
200
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
0.01
1.94
0.48
5.11
7.21
272.55
0.52
2 Months
4 Months
6 Months
9 Months
Time Post DU Pellet Implantation

Figure 39

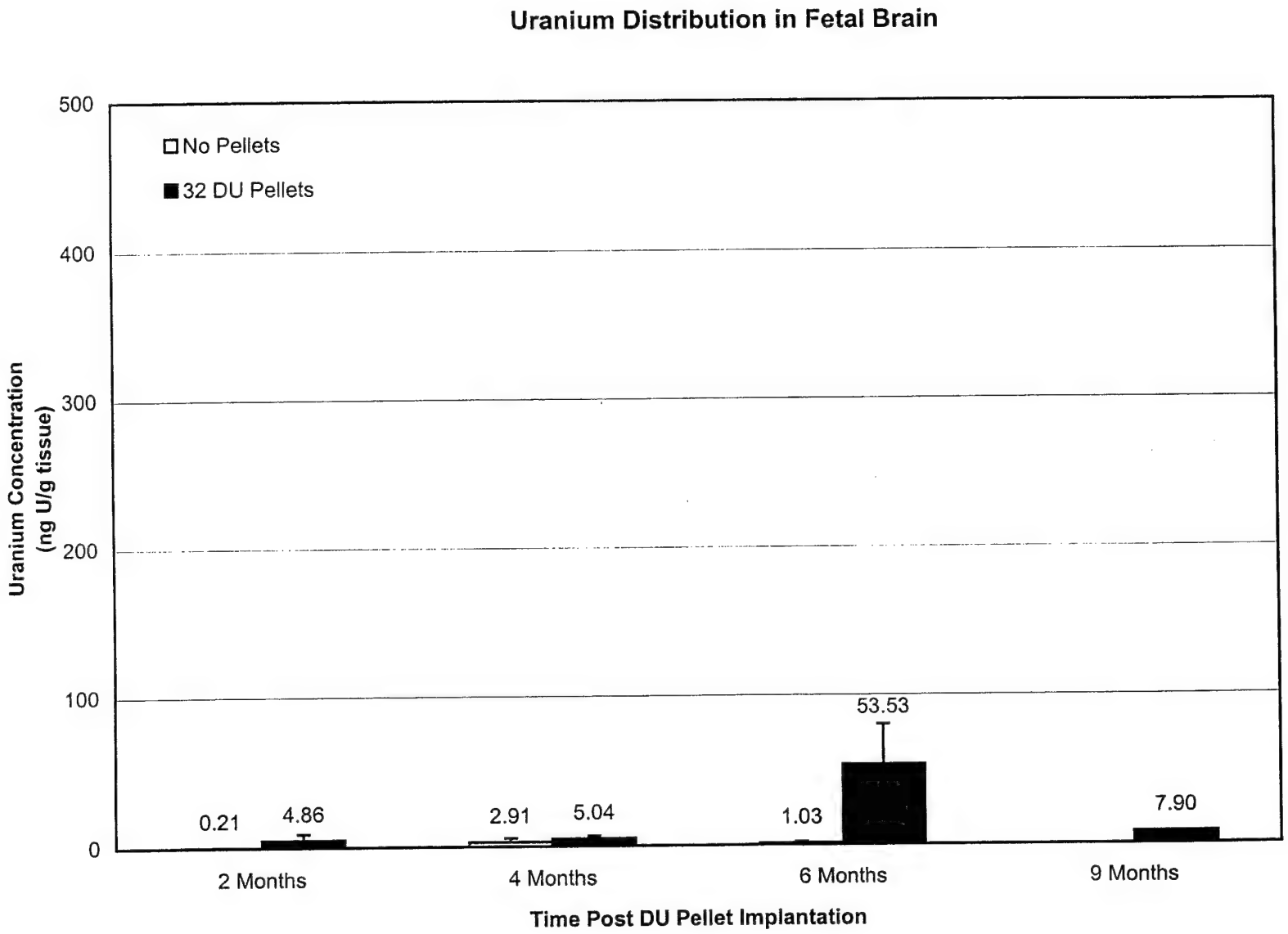

Uranium Distribution in Fetal Brain
500
400
300
200
100
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
0.21
4.86
2.91
5.04
1.03
53.53
7.90
2 Months
4 Months
6 Months
9 Months
Time Post DU Pellet Implantation

Figure 40

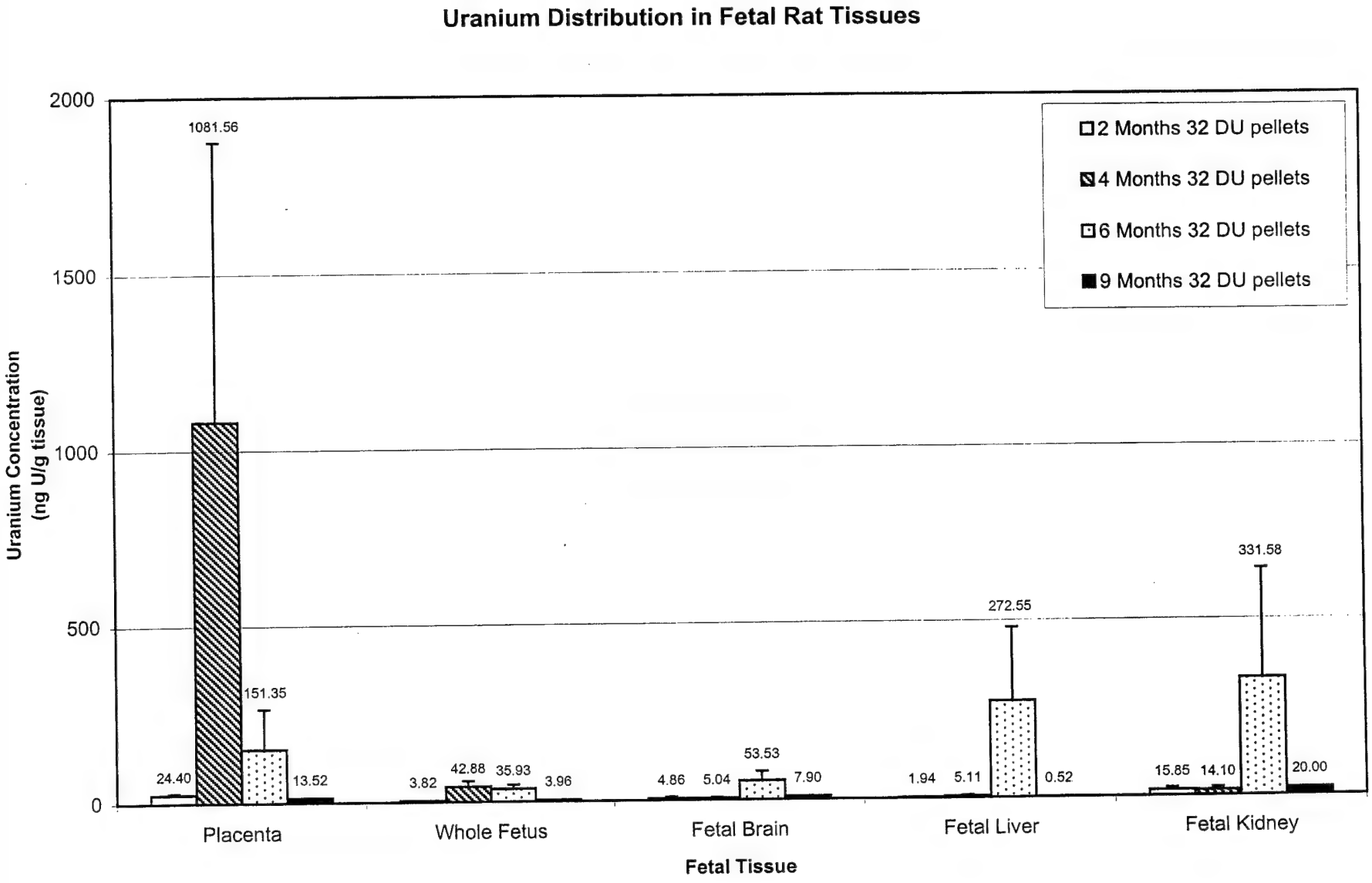

Uranium Distribution in Fetal Rat Tissues
2000
1500
1000
500
0
Uranium Concentration (ng U/g tissue)
2 Months 32 DU pellets
4 Months 32 DU pellets
6 Months 32 DU pellets
9 Months 32 DU pellets
1081.56
151.35
24.40
13.52
3.82
42.88
35.93
3.96
4.86
5.04
53.53
7.90
1.94
5.11
272.55
0.52
15.85
14.10
331.58
20.00
Placenta
Whole Fetus
Fetal Brain
Fetal Liver
Fetal Kidney
Fetal Tissue

Figure 41

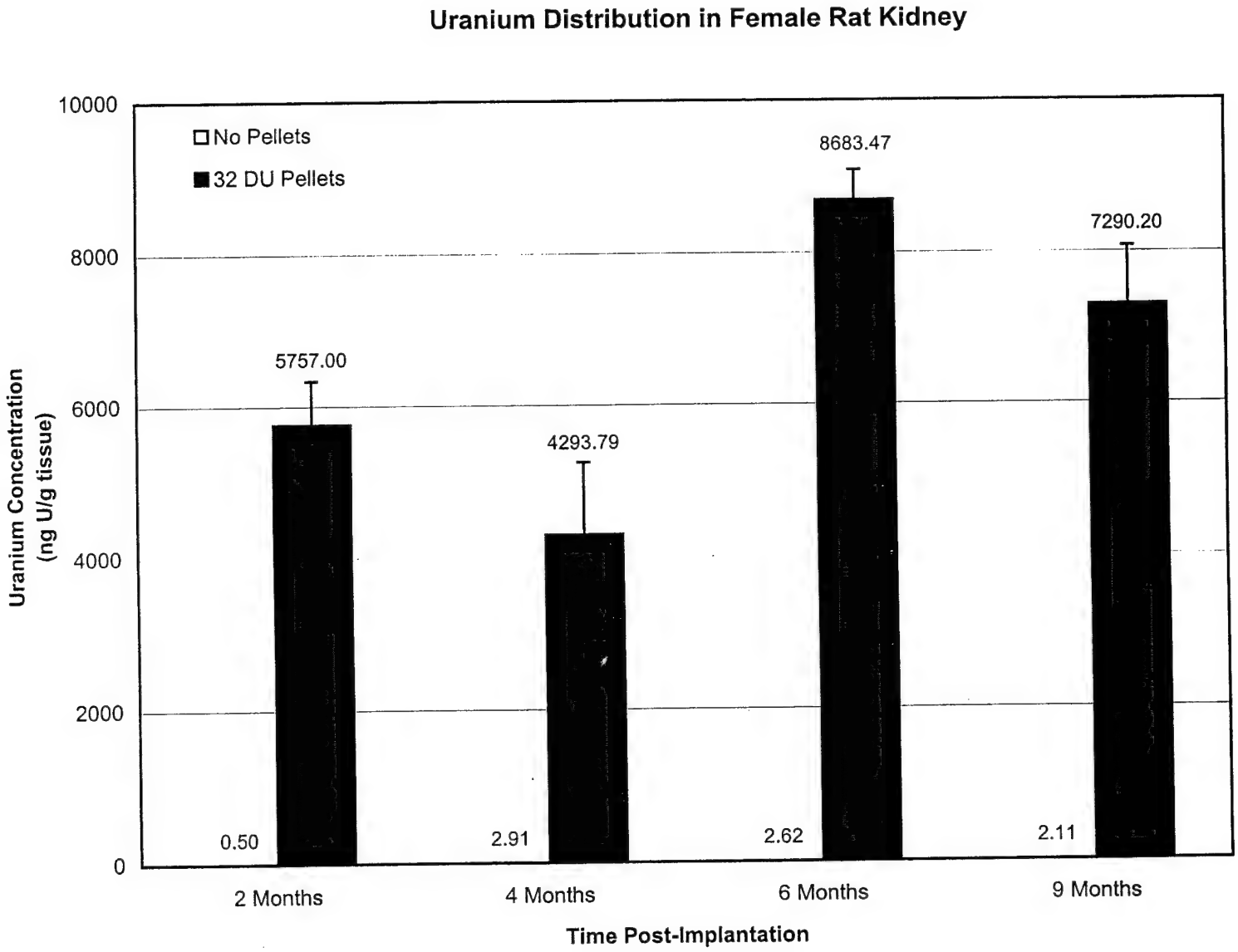

Uranium Distribution in Female Rat Kidney
10000
8000
6000
4000
2000
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
5757.00
4293.79
8683.47
7290.20
0.50
2.91
2.62
2.11
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 42

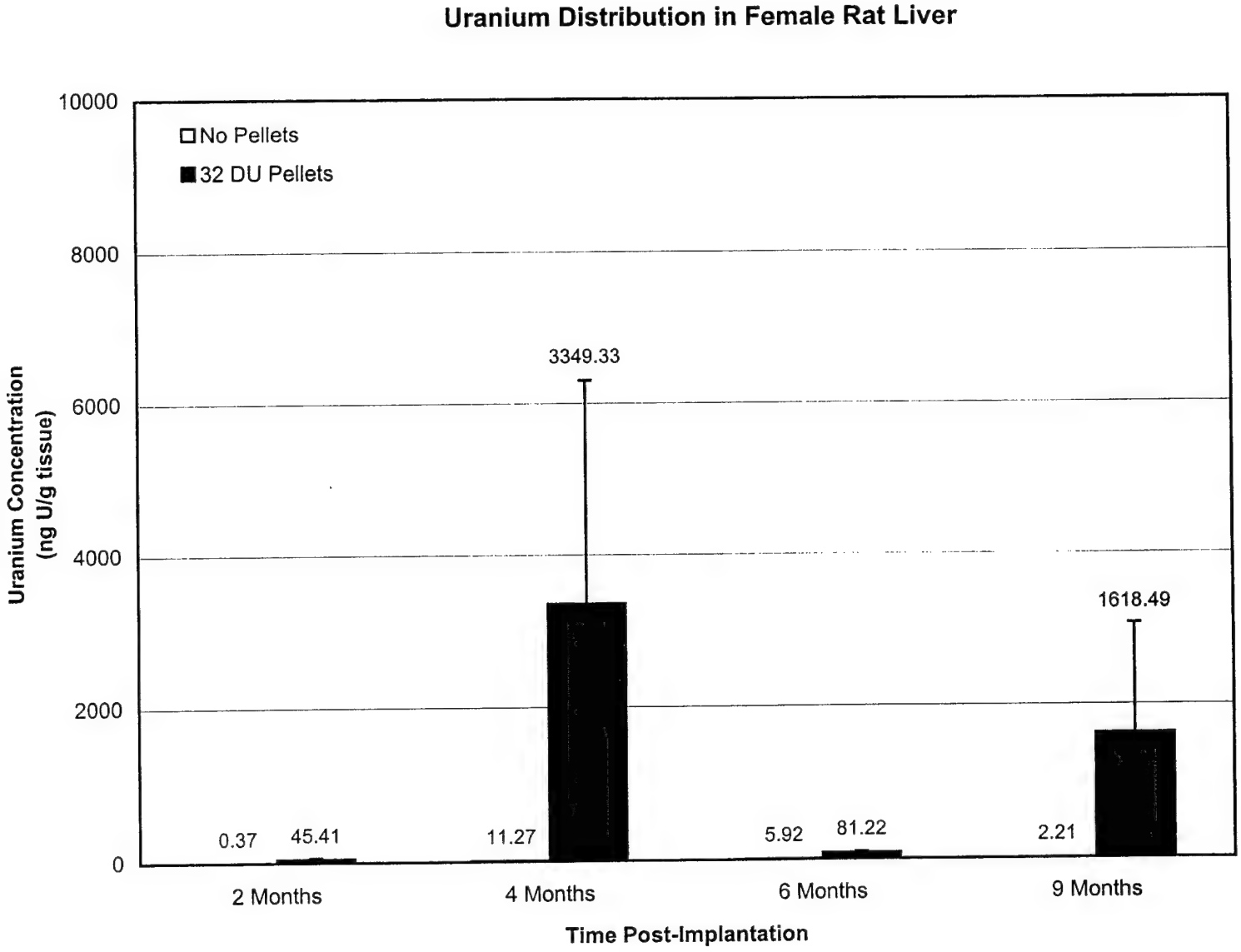

Uranium Distribution in Female Rat Liver
10000
8000
6000
4000
2000
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
3349.33
1618.49
0.37
45.41
11.27
5.92
81.22
2.21
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 43

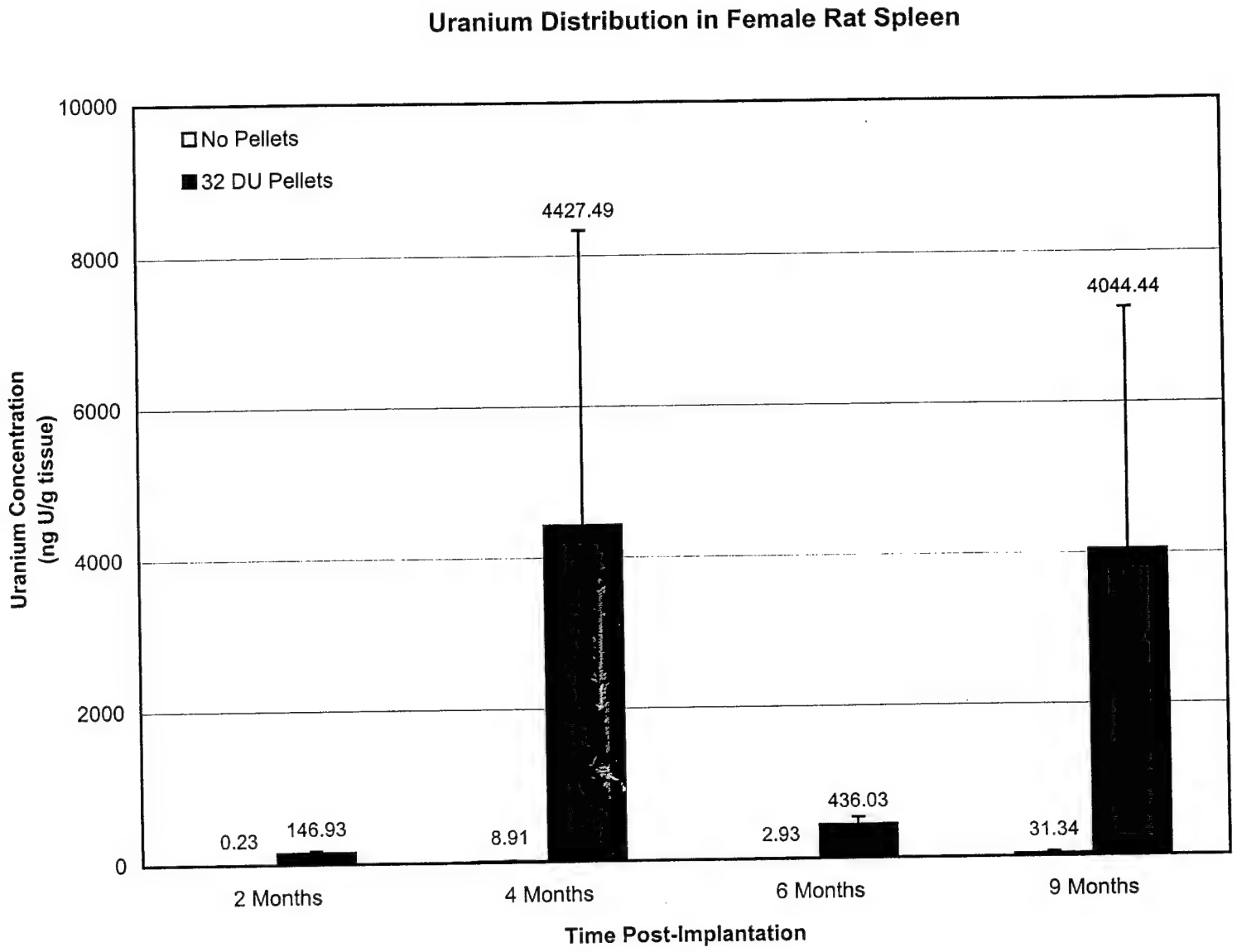

Uranium Distribution in Female Rat Spleen
10000
8000
6000
4000
2000
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
4427.49
4044.44
436.03
0.23
146.93
8.91
2.93
31.34
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 44

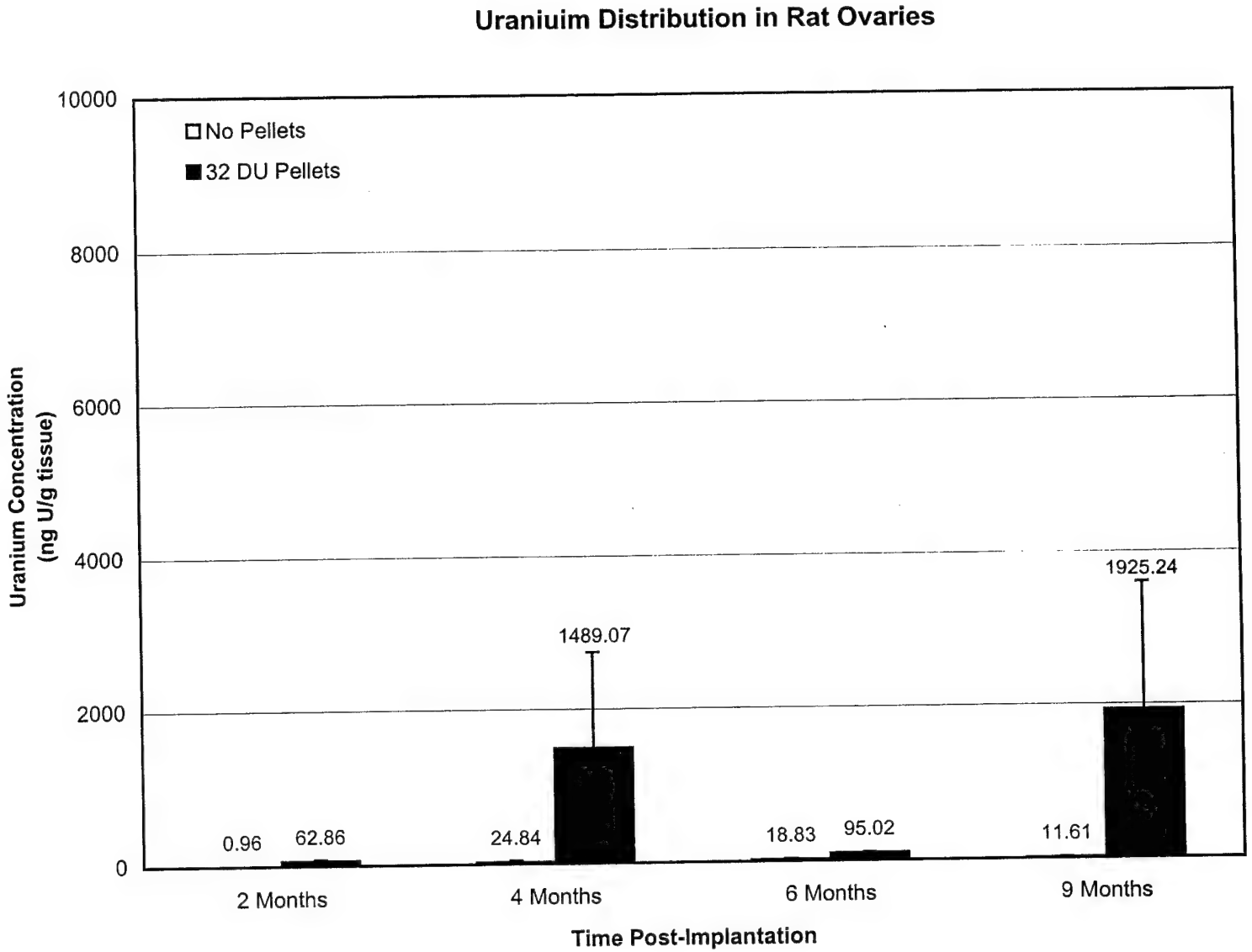

Uraniuim Distribution in Rat Ovaries
10000
8000
6000
4000
2000
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
0.96
62.86
24.84
1489.07
18.83
95.02
11.61
1925.24
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 45

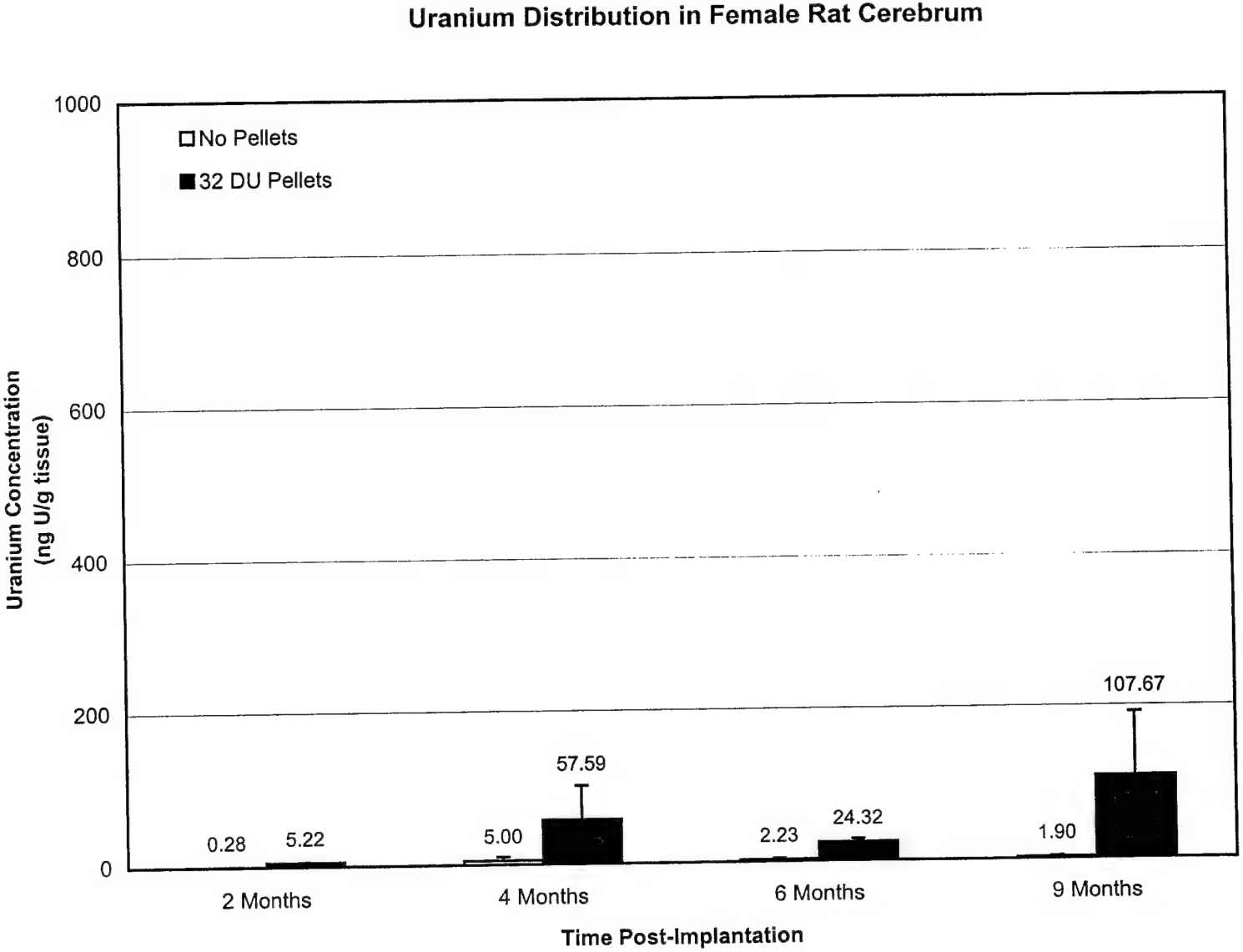

Uranium Distribution in Female Rat Cerebrum
1000
800
600
400
200
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
0.28
5.22
5.00
57.59
2.23
24.32
1.90
107.67
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 46

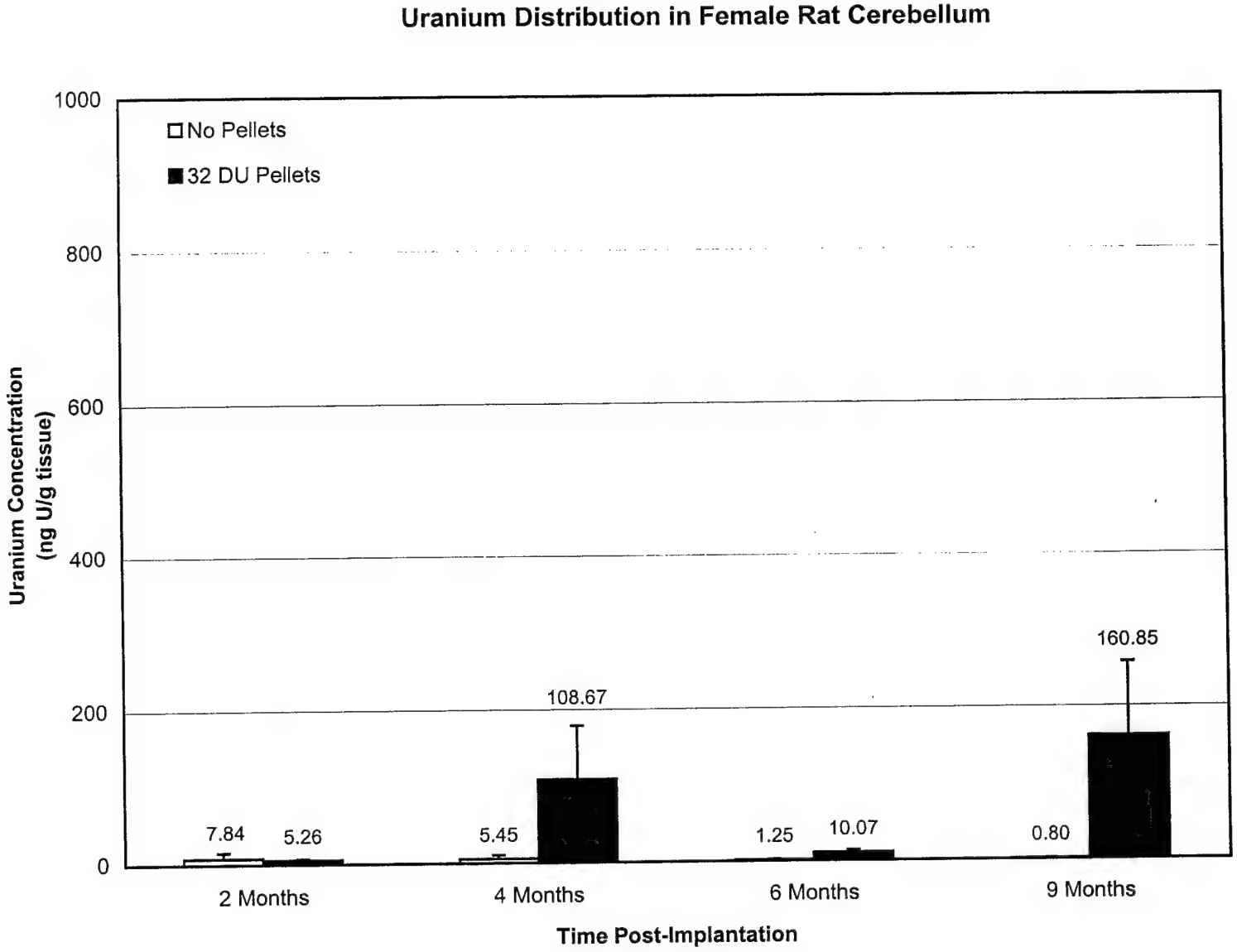

Uranium Distribution in Female Rat Cerebellum
1000
800
600
400
200
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
7.84
5.26
5.45
108.67
1.25
10.07
0.80
160.85
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 47

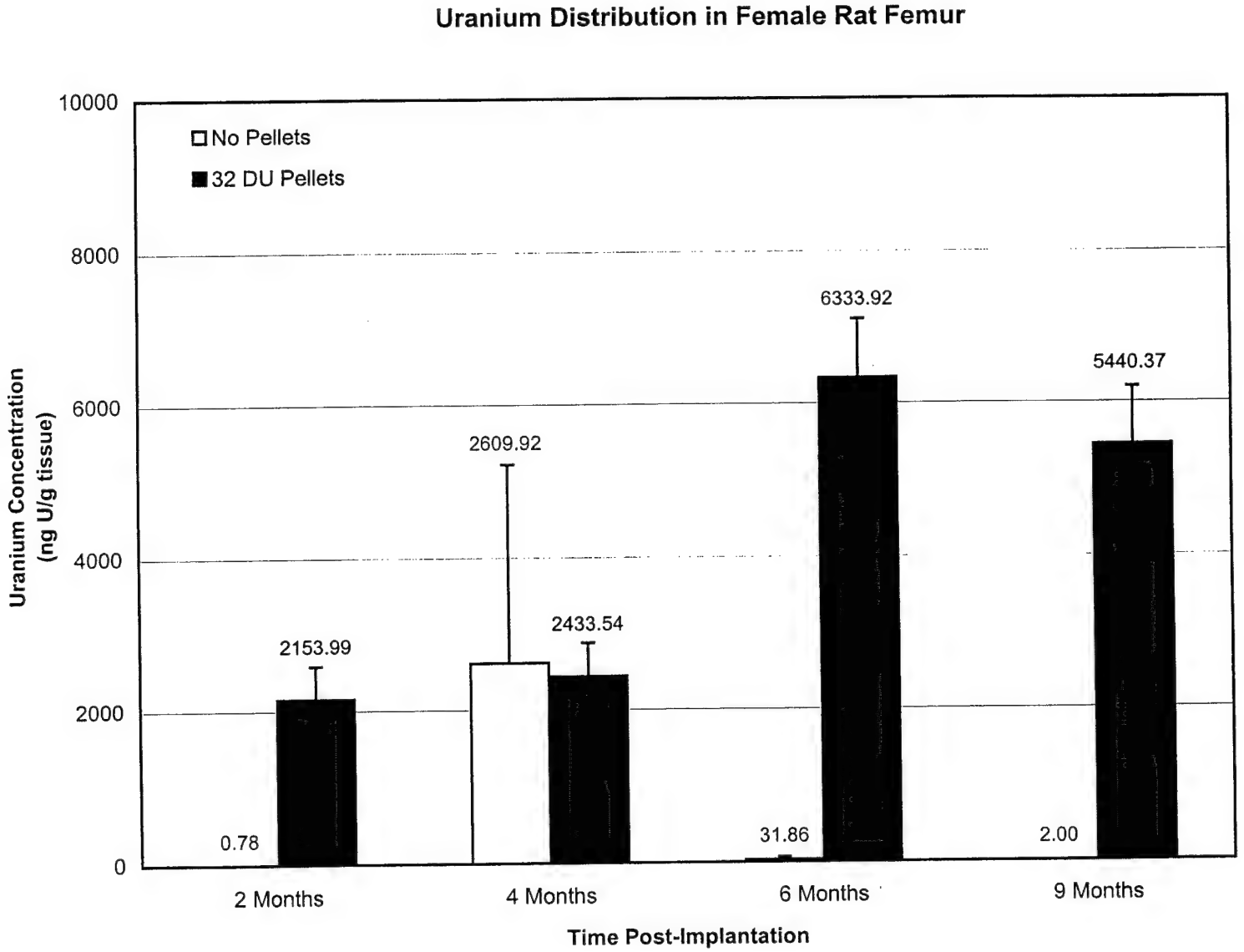

Uranium Distribution in Female Rat Femur
10000
8000
6000
4000
2000
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
0.78
2153.99
2609.92
2433.54
31.86
6333.92
2.00
5440.37
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 48

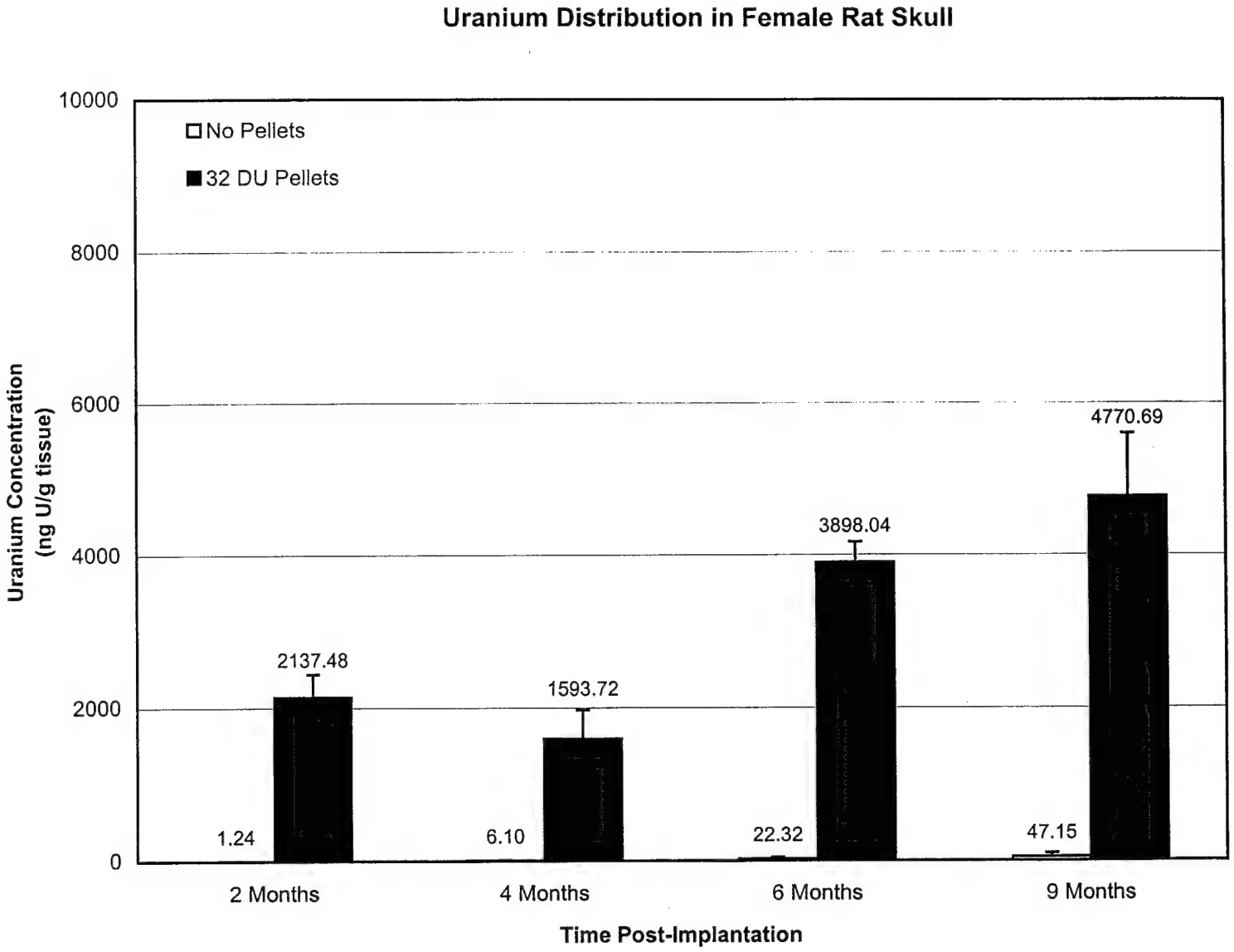

Uranium Distribution in Female Rat Skull
10000
8000
6000
4000
2000
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
1.24
2137.48
6.10
1593.72
22.32
3898.04
47.15
4770.69
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 49

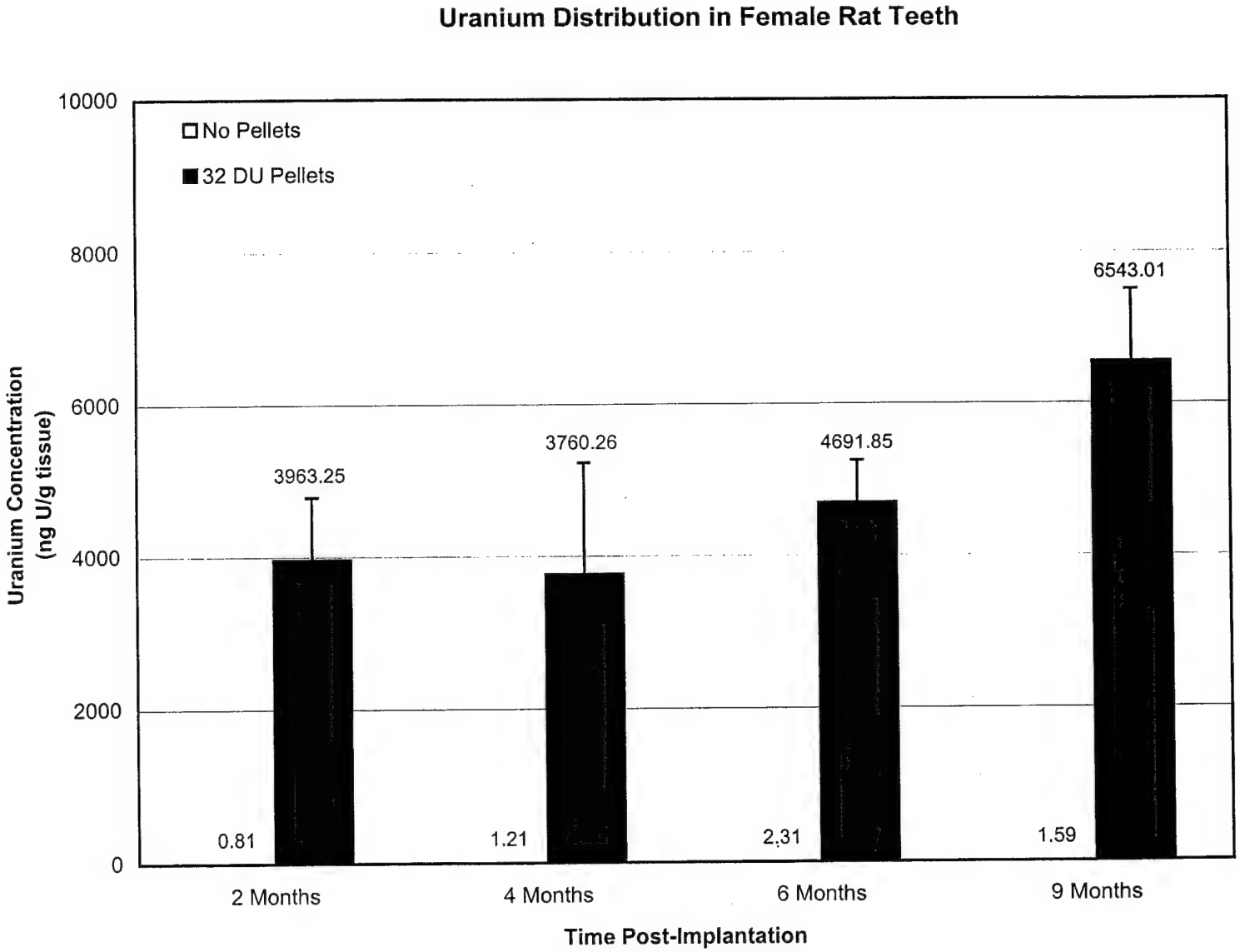

Uranium Distribution in Female Rat Teeth
10000
8000
6000
4000
2000
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
3963.25
3760.26
4691.85
6543.01
0.81
1.21
2.31
1.59
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 50

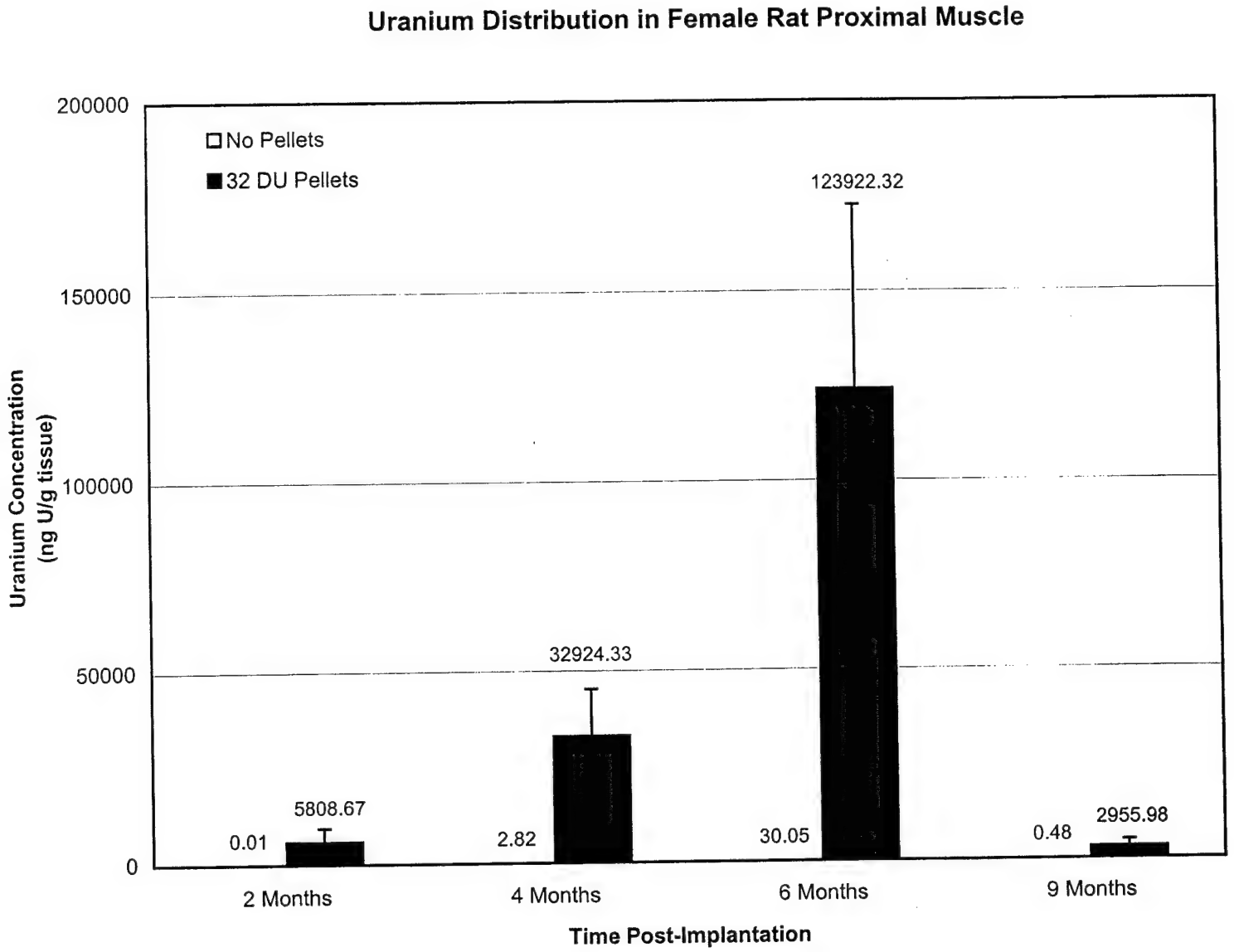

Uranium Distribution in Female Rat Proximal Muscle
200000
150000
100000
50000
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
0.01
5808.67
2.82
32924.33
30.05
123922.32
0.48
2955.98
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 51

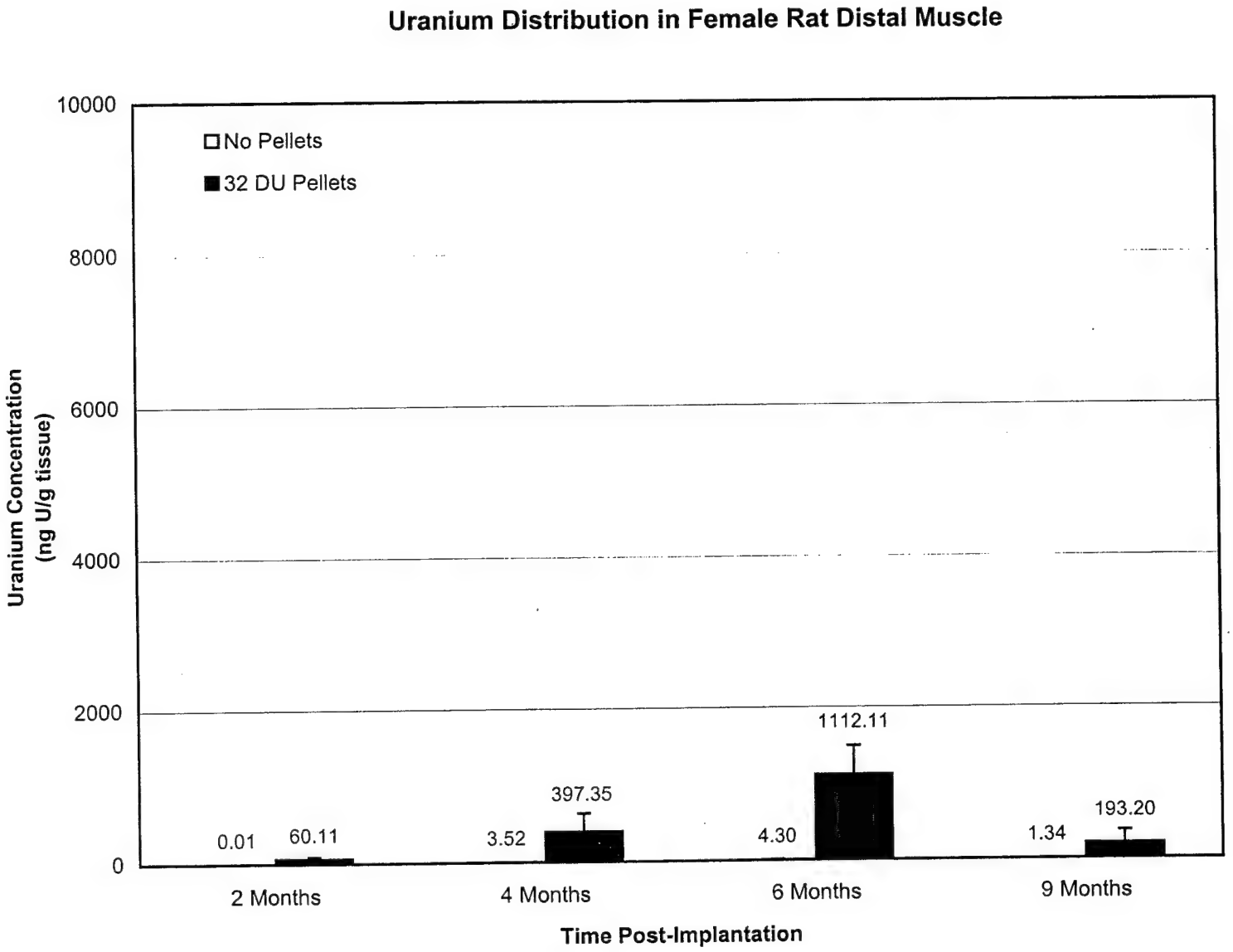

Uranium Distribution in Female Rat Distal Muscle
10000
8000
6000
4000
2000
0
No Pellets
32 DU Pellets
Uranium Concentration (ng U/g tissue)
0.01
60.11
3.52
397.35
4.30
1112.11
1.34
193.20
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 52

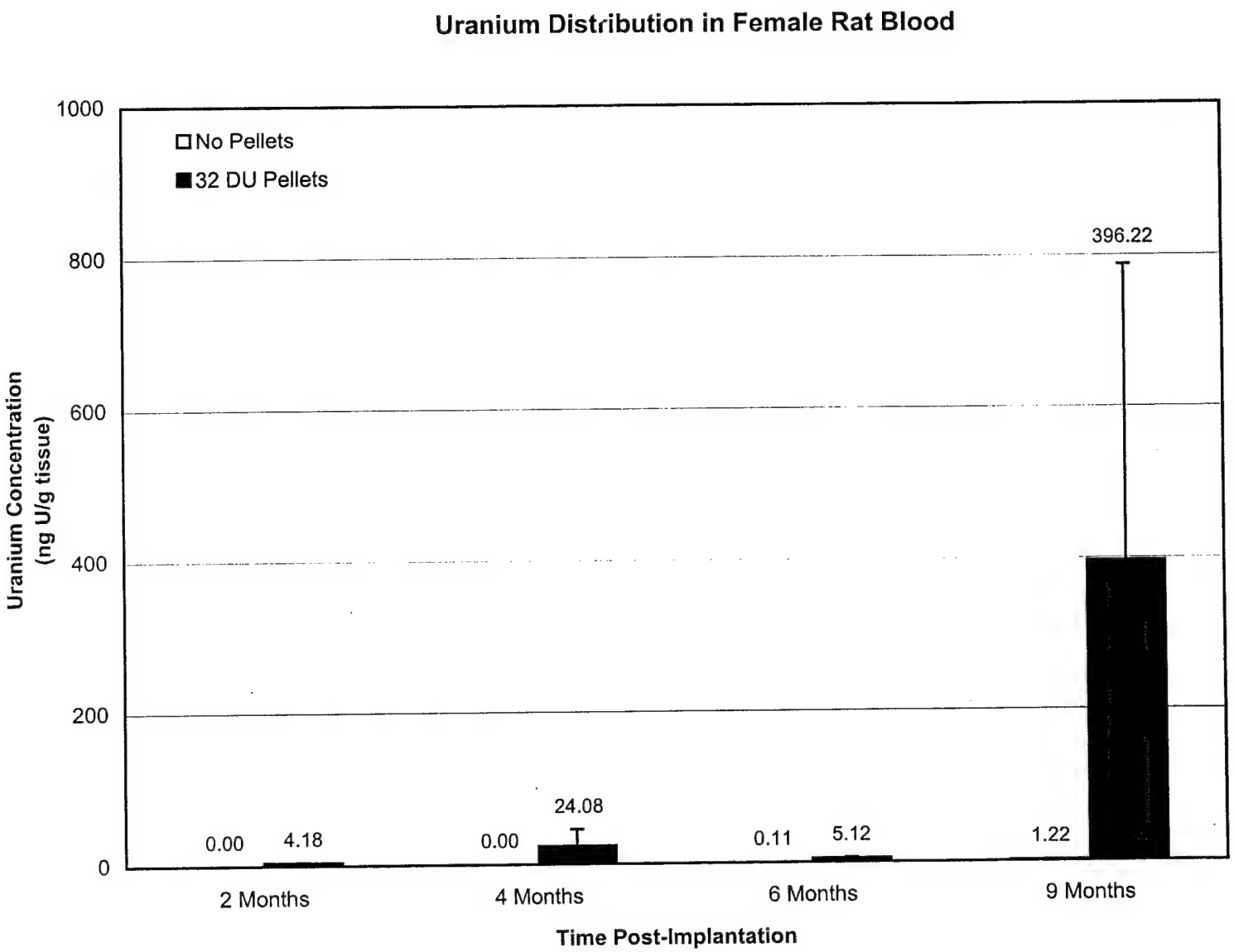

Uranium Distribution in Female Rat Blood
No Pellets
32 DU Pellets
1000
800
600
400
200
0
Uranium Concentration (ng U/g tissue)
0.00
4.18
0.00
24.08
0.11
5.12
1.22
396.22
2 Months
4 Months
6 Months
9 Months
Time Post-Implantation

Figure 53

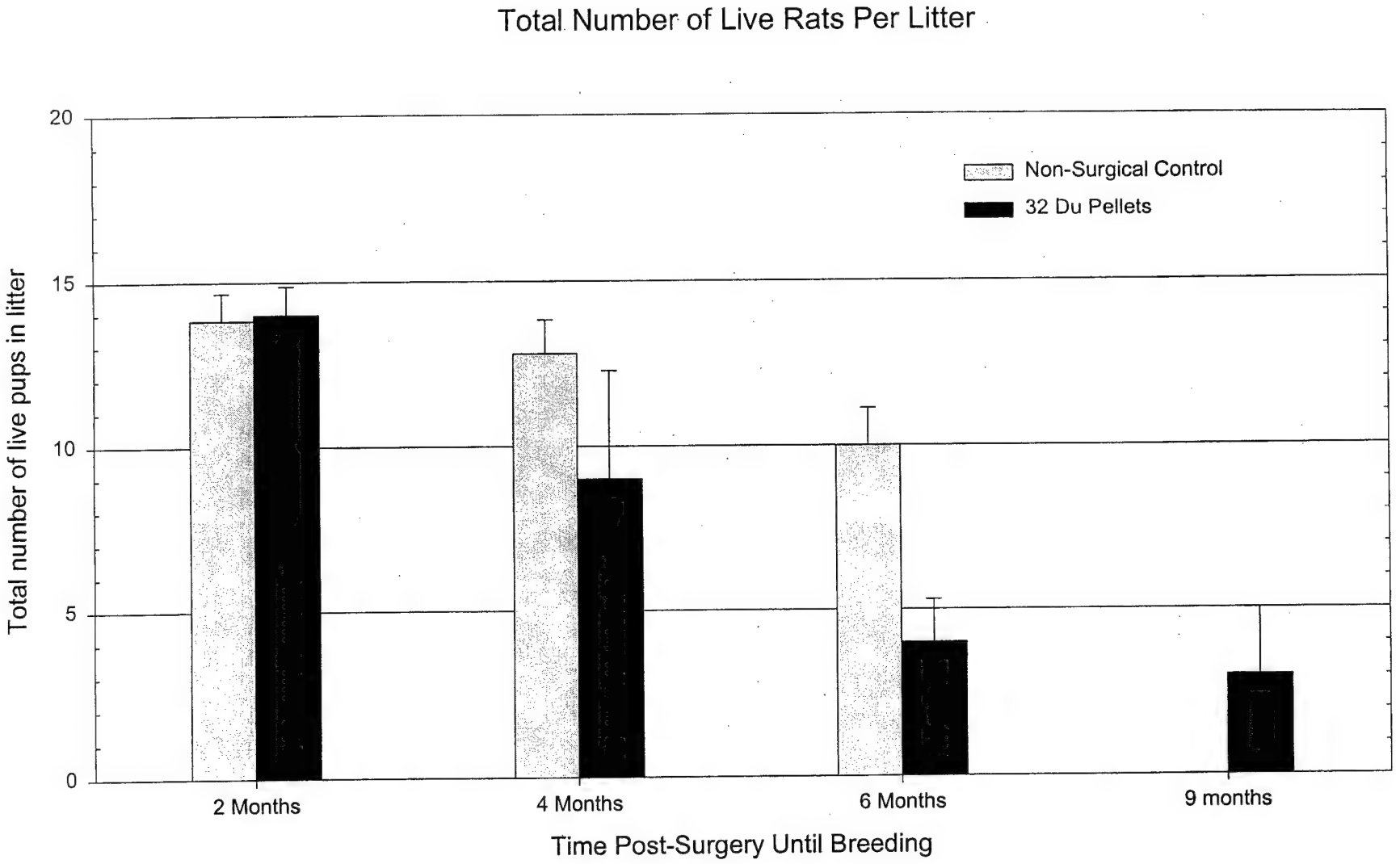

Total Number of Live Rats Per Litter
Non-Surgical Control
32 Du Pellets
Total number of live pups in litter
20
15
10
5
0
2 Months
4 Months
6 Months
9 months
Time Post-Surgery Until Breeding

Figure 54

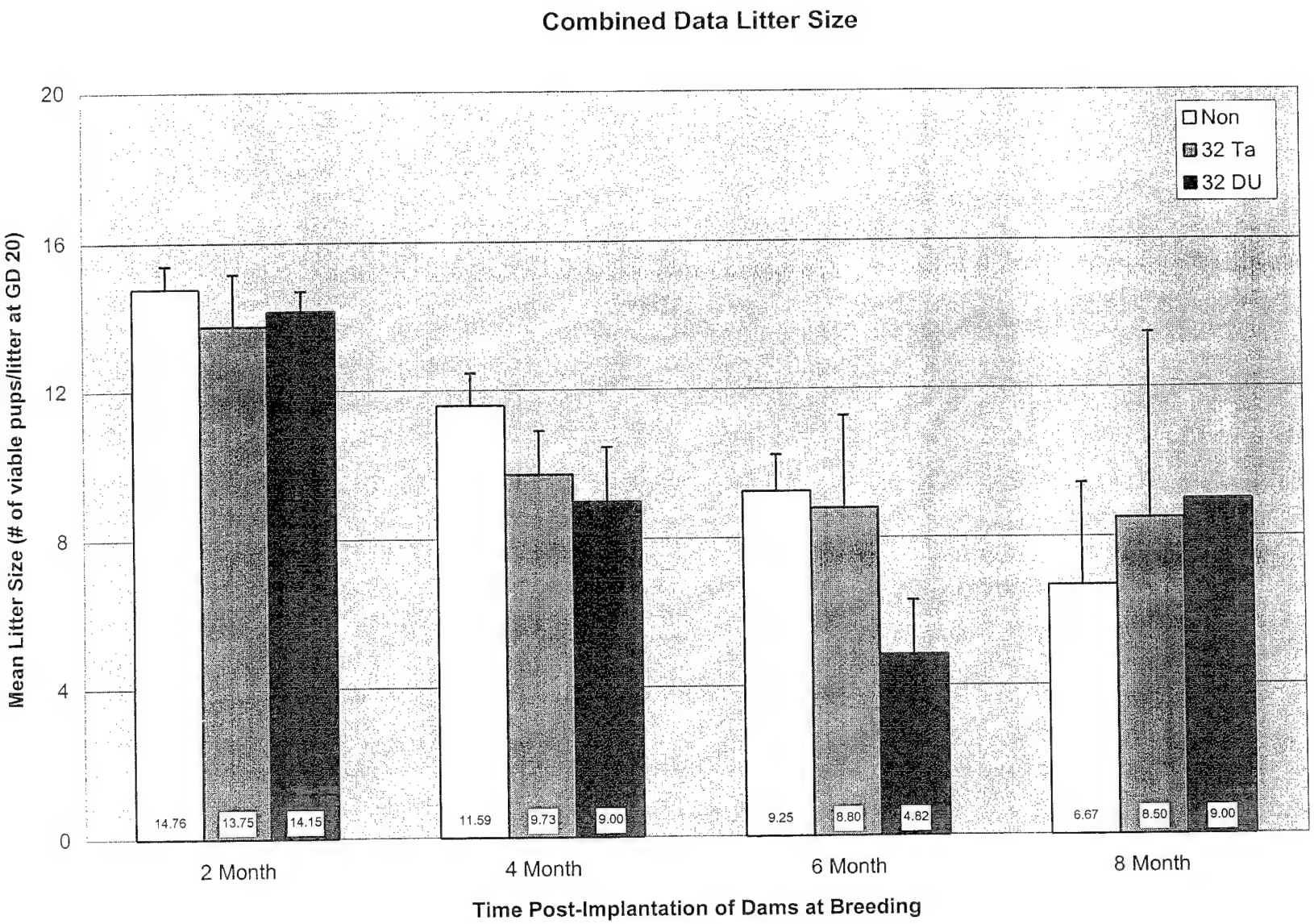

Combined Data Litter Size
Mean Litter Size (# of viable pups/litter at GD 20)
20
16
12
8
4
0
Non
32 Ta
32 DU
14.76
13.75
14.15
11.59
9.73
9.00
9.25
8.80
4.82
6.67
8.50
9.00
2 Month
4 Month
6 Month
8 Month
Time Post-Implantation of Dams at Breeding

Figure 55

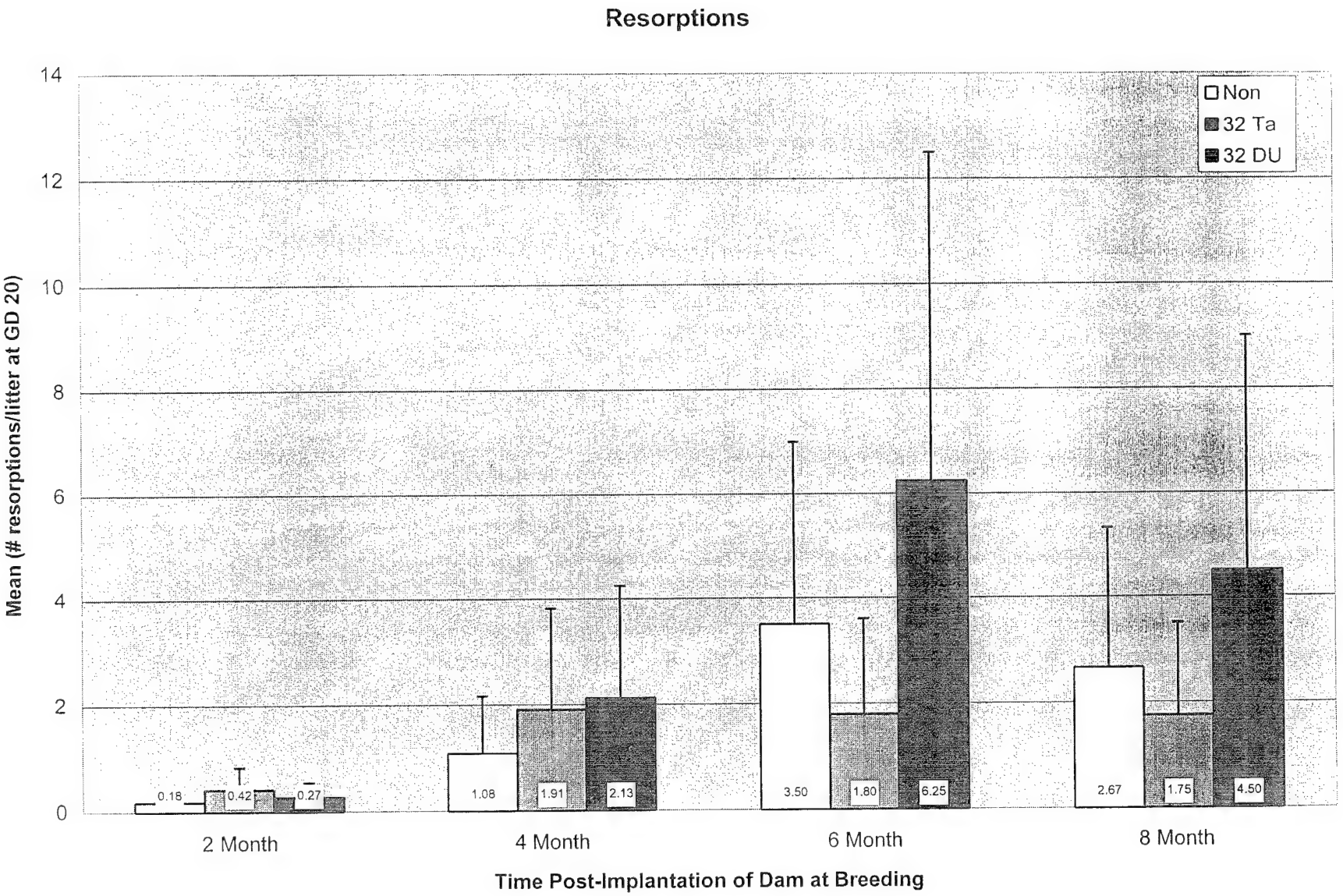

Resorptions
Non
32 Ta
32 DU
Mean (# resorptions/litter at GD 20)
0
2
4
6
8
10
12
14
0.18
0.42
0.27
1.08
1.91
2.13
3.50
1.80
6.25
2.67
1.75
4.50
2 Month
4 Month
6 Month
8 Month
Time Post-Implantation of Dam at Breeding

Figure 56

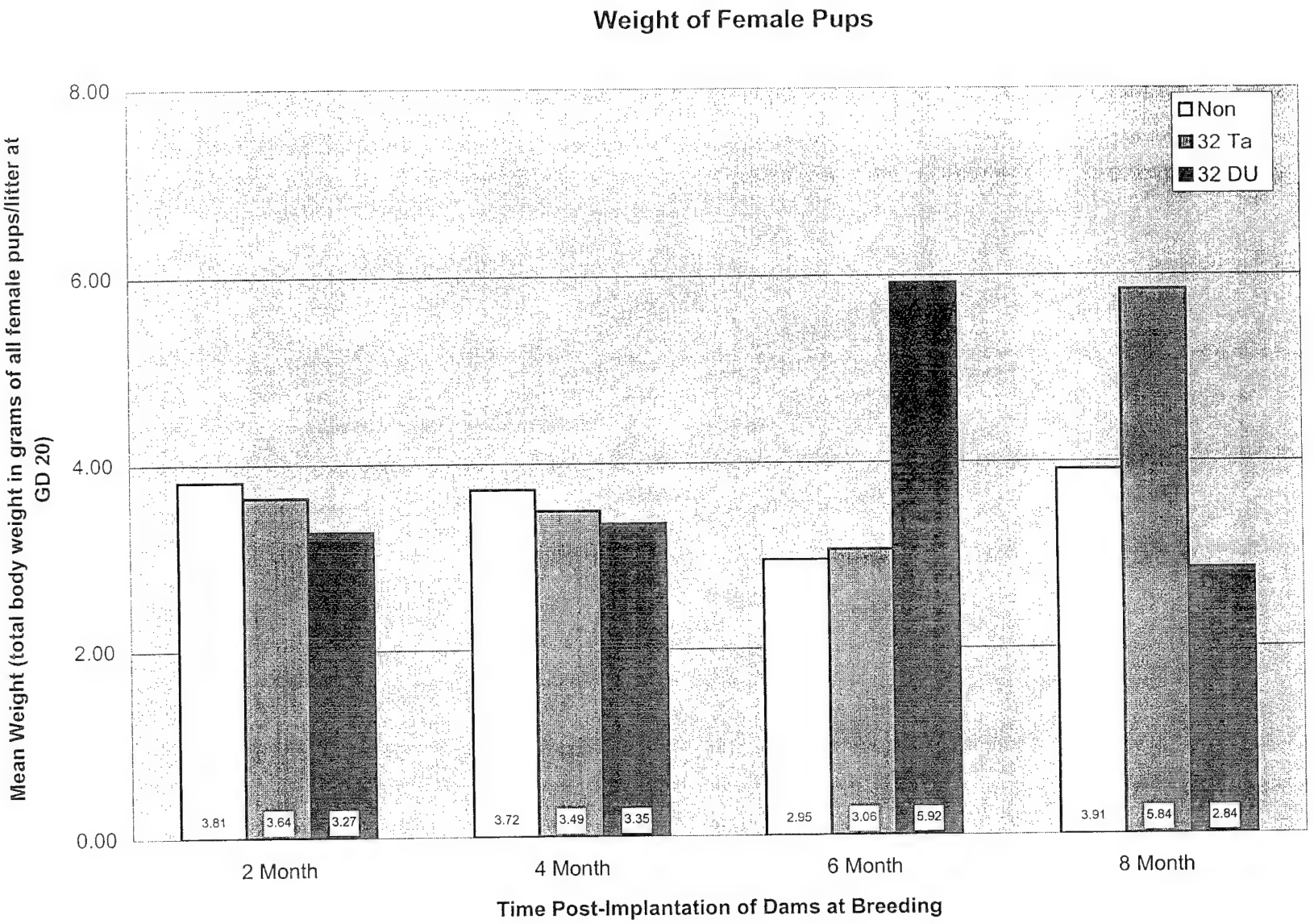

Weight of Female Pups
Mean Weight (total body weight in grams of all female pups/litter at GD 20)
8.00
6.00
4.00
2.00
0.00
Non
32 Ta
32 DU
3.81
3.64
3.27
3.72
3.49
3.35
2.95
3.06
5.92
3.91
5.84
2.84
2 Month
4 Month
6 Month
8 Month
Time Post-Implantation of Dams at Breeding

Figure 57

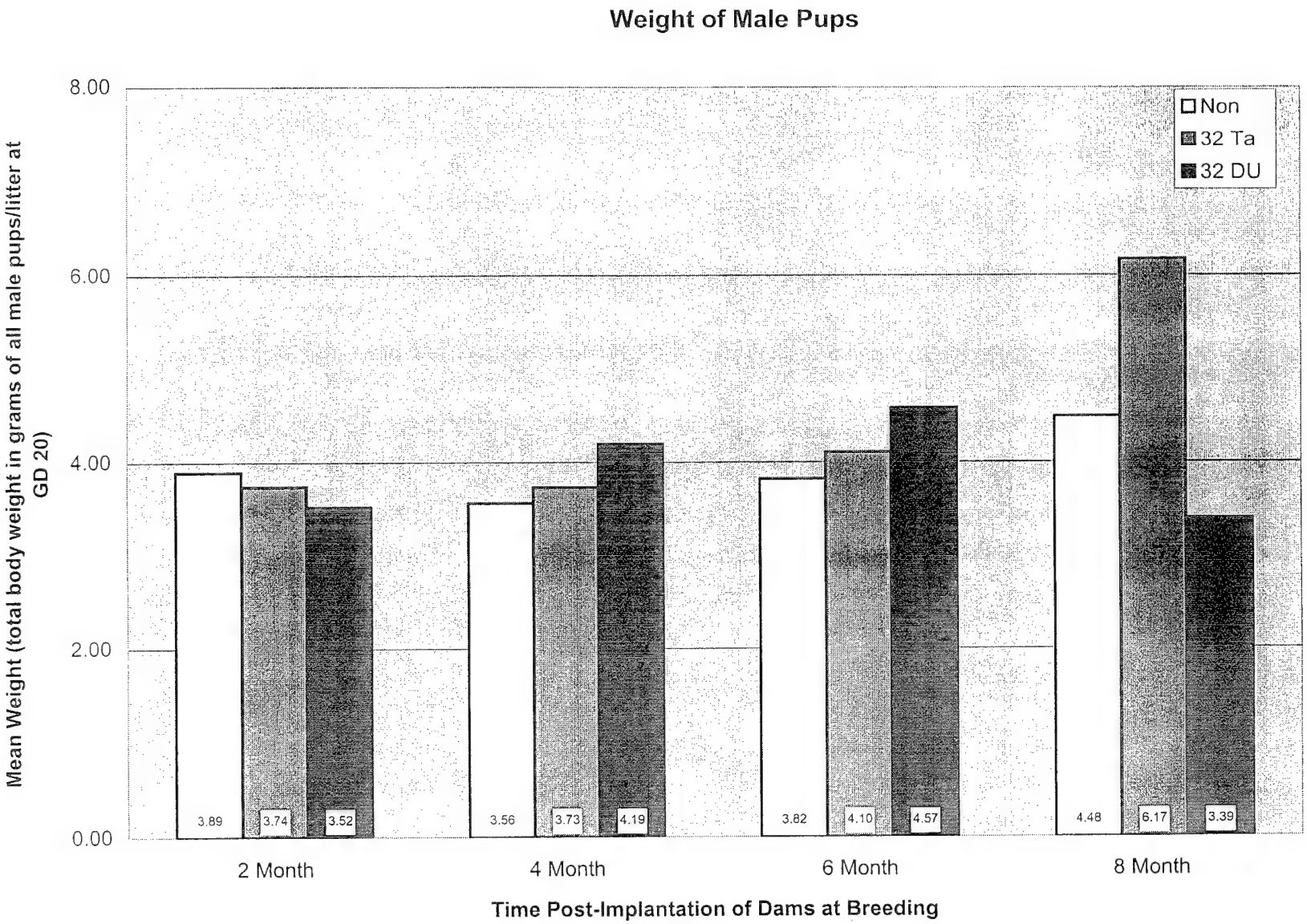

Weight of Male Pups
Mean Weight (total body weight in grams of all male pups/litter at GD 20)
8.00
6.00
4.00
2.00
0.00
Non
32 Ta
32 DU
3.89
3.74
3.52
3.56
3.73
4.19
3.82
4.10
4.57
4.48
6.17
3.39
2 Month
4 Month
6 Month
8 Month
Time Post-Implantation of Dams at Breeding

Figure 58

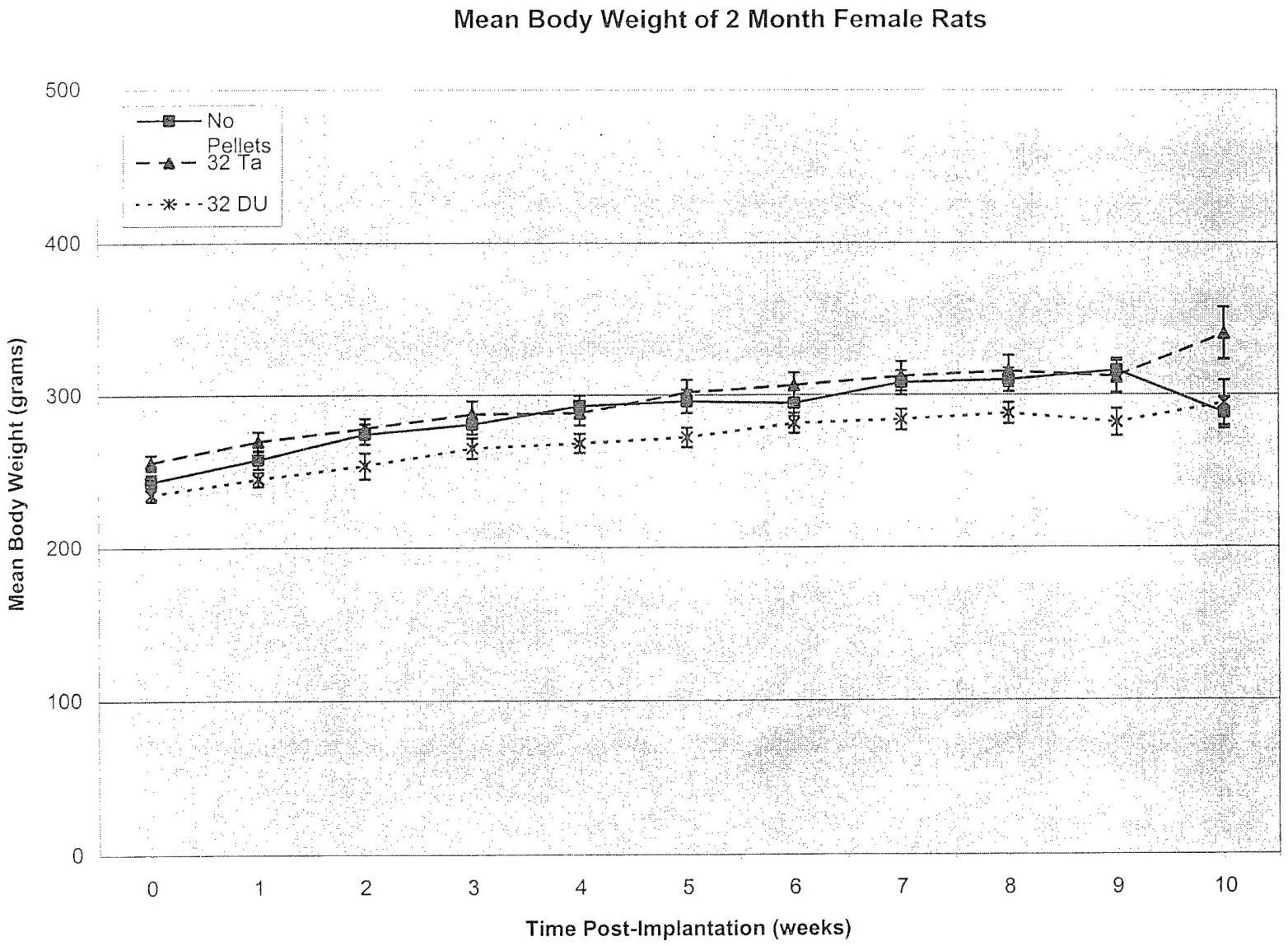

Mean Body Weight of 2 Month Female Rats
No Pellets
32 Ta
32 DU
Mean Body Weight (grams)
500
400
300
200
100
0
0
1
2
3
4
5
6
7
8
9
10
Time Post-Implantation (weeks)

Figure 59

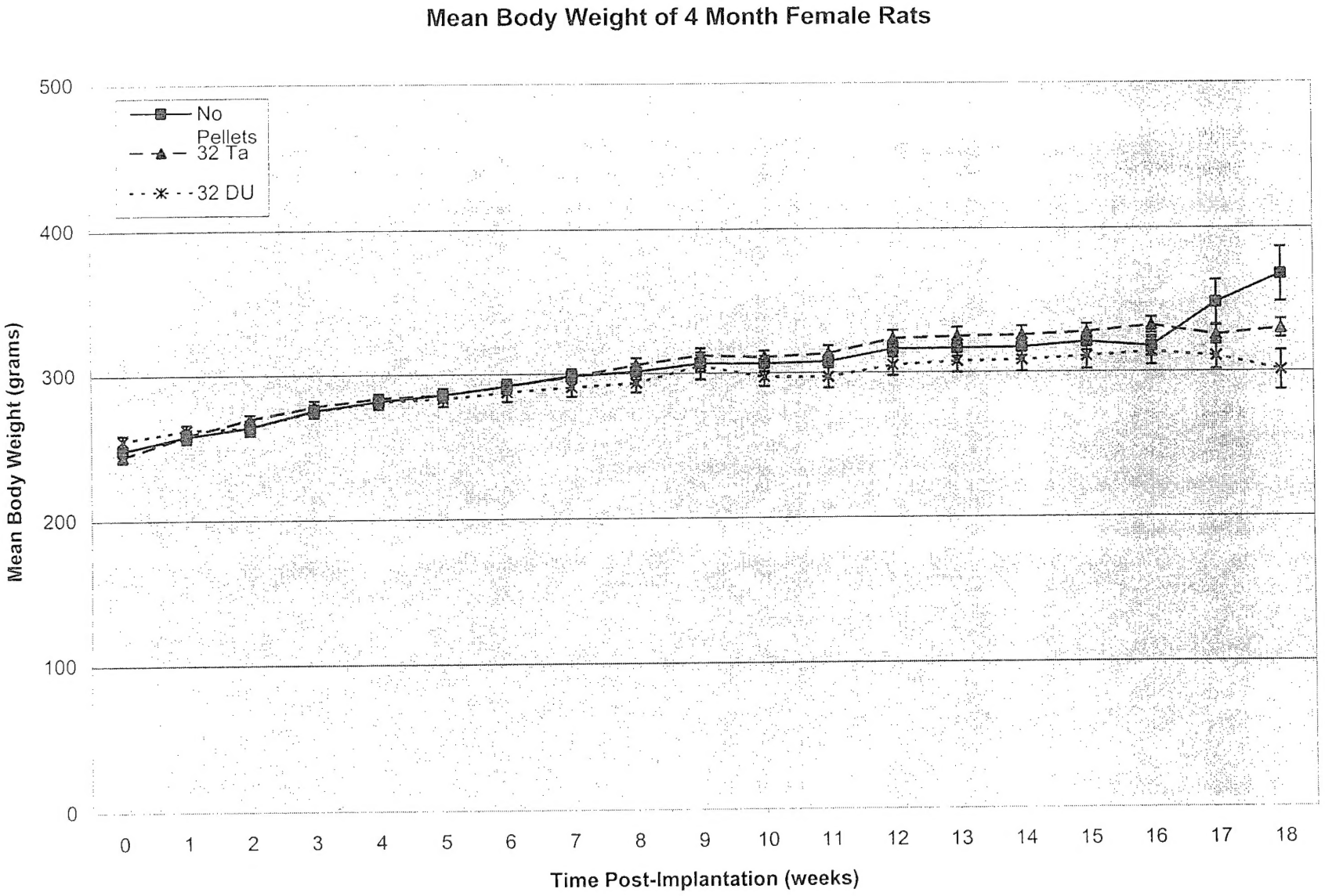

Mean Body Weight of 4 Month Female Rats
No Pellets
32 Ta
32 DU
Mean Body Weight (grams)
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Time Post-Implantation (weeks)

Figure 60

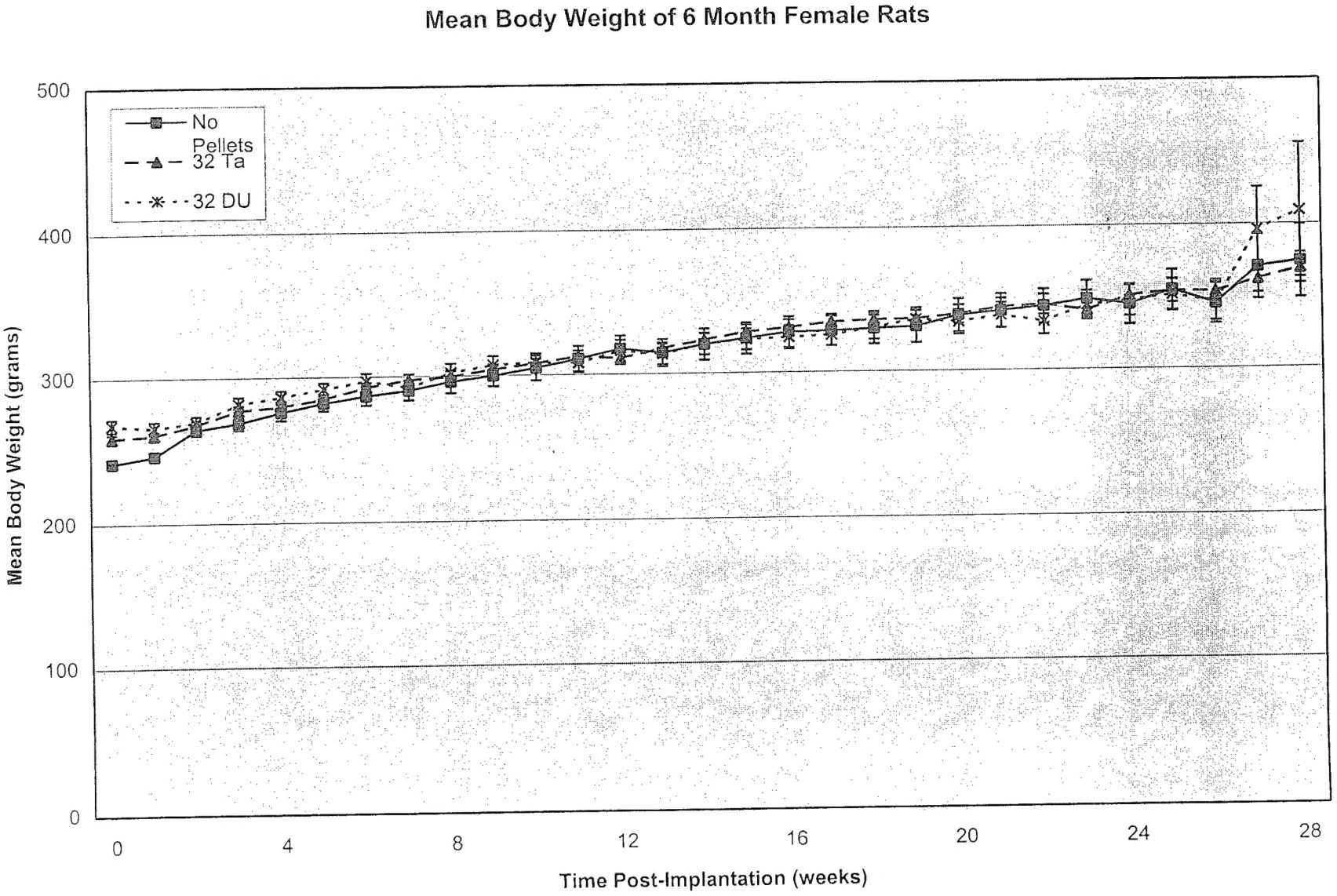
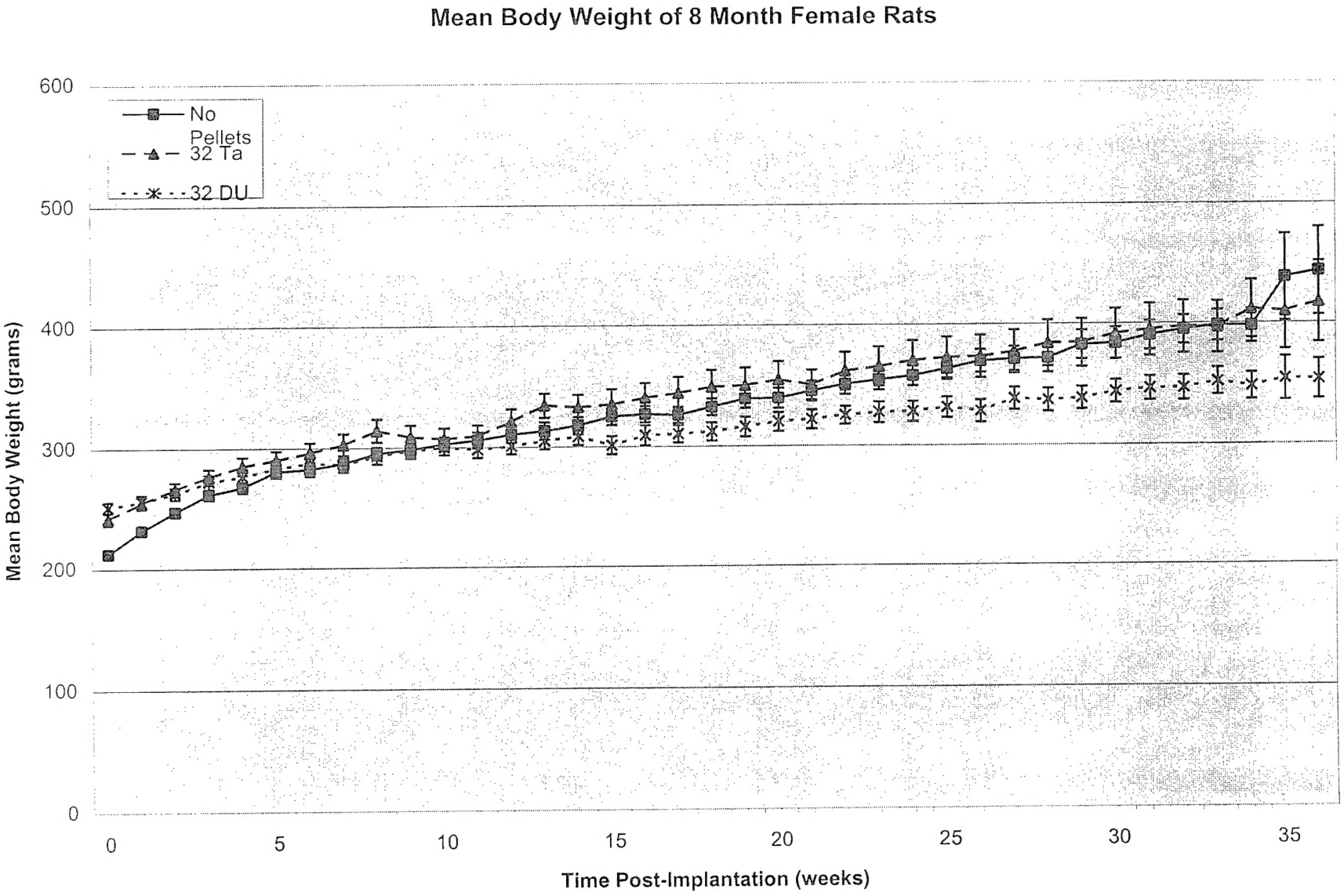

Mean Body Weight of 6 Month Female Rats
No Pellets
32 Ta
32 DU
Mean Body Weight (grams)
500
400
300
200
100
0
0
4
8
12
16
20
24
28
Time Post-Implantation (weeks)

Figure 61


Mean Body Weight of 8 Month Female Rats
600
500
400
300
200
100
0
No Pellets
32 Ta
32 DU
Mean Body Weight (grams)
0
5
10
15
20
25
30
35
Time Post-Implantation (weeks)